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Sex-dependent Regulation of Social Reward by Oxytocin Receptors in the Ventral Tegmental Area: an Inverted U Hypothesis

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SEX-DEPENDENT REGULATION OF SOCIAL REWARD BY OXYTOCIN RECEPTORS IN THE VENTRAL TEGMENTAL AREA: AN INVERTED U HYPOTHESIS

by

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Under the Direction of H. ELLIOTT ALBERS PhD

ABSTRACT

Social reward is critical for social relationships, and yet we know little about the characteristics of social interactions that are rewarding or the neural mechanisms underlying that reward. Furthermore, sex differences in the neural mechanisms mediating social reward likely contribute to the sex differences in the prevalence and predisposition to many psychiatric disorders. Here, using a variety of behavioral, pharmacological, neuroendocrine and molecular approaches we investigate the behavioral characteristics underlying the rewarding properties of same-sex social interactions and the sex-dependent role of the oxytocin system in regulating the magnitude and valence of social reward. We found 1) that there may be an inverted U shaped dose response relationship between the duration of social interaction and social reward value, 2)

females find same-sex social interactions more rewarding than males and 3) the OT system is necessary for social reward, in both males and females and depending on the social context "social dose", activation of OTRs in the VTA can increase social reward in males, but have the opposite effect, decrease social reward in females. Collectively, these studies provide support for the hypothesis there is an inverted U relationship between the duration of social interaction and social reward, and that females may be more sensitive to the rewarding effects of social interactions. Furthermore, the OT system mediates social reward in males and females, and more specifically, OT can have the opposite effect on social reward in males and females. In conclusion, understanding these sex differences in social reward processing may be essential for understanding the sex differences in the prevalence of many psychiatric disorders and the development of sex-specific treatments of neuropsychiatric disorders.

INDEX WORDS: oxytocin receptors, social reward, sex differences, ventral tegmental area, dopamine, psychiatric disorders

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DEDICATION

This dissertation is dedicated to all the friends, family and colleagues that have been a part of my life. However, I want to particularly highlight and thank my loving father (John), mother (Laura), and sister (Katherine); and my loving pets Brady and Lily.

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LIST OF ABBREVIATIONS

ABS	antibody serum
ABSL	animal biosafety level
ANOVA	analysis of variance
AP	anterior-posterior
AVP	vasopressin
BOLD	blood-oxygen-level dependent imaging
C	Celsius
CeA	central nucleus of the amygdala
cm	centimeter
CPP	Conditioned Place Preference
CSF	cerebrospinal fluid
DA	dopamine
DOPAC	3,4-Dihydroxyphenylacetic acid
DV	dorsal-ventral
ED	Extinction Day
fMRI	function magnetic resonance imaging
g	grams
hr	hour
ICC	immunocytochemistry
IP	intraperitoneal
ir	immunoreactivity
IU	international units

kg	kilogram
LD	light dark cycle
mg	milligram
ml	milliliter
mm	millimeter
min	minute
ML	medial-lateral
µg	microgram
µl	microliter
µM	micromolar
µm	micrometer
NAc	nucleus accumbens
NDS	normal donkey serum
nl	nanoliter
OSP	Operant Social Preference
OT	oxytocin
OTA	selective oxytocin receptor antagonist
OTR	oxytocin receptor
oz	ounce
PBS	phosphate-buffered saline
PVN	paraventricular nucleus
PW	progressive weights
SD	Social Day

sec	second
SEM	standard error of the mean
SON	supraoptic nucleus
TBS	Tris-buffered saline
TGOT	selective oxytocin receptor agonist
TH	tyrosine hydroxylase
V1aR	vasopressin receptor
VTA	ventral tegmental area

1 CHAPTER ONE: GENERAL INTRODUCTION

1.1 Overview

The rewarding properties of social interactions are critical for the expression of adaptive social behaviors, including the development of social relationships in most species (Darwin, 1859, Skinner, 1938, Oliveira et al., 1998, Krach et al., 2010, Pettinger et al., 2011). In humans, deficits in the rewarding properties of social stimuli likely contribute to many psychiatric disorders (Dichter et al., 2012, Stravropoulos & Carver, 2013, Foulkes et al., 2015, Novacek et al., 2016). Furthermore, potential sex differences in the neural mechanisms underlying social reward likely contribute to the well-known sex differences in the prevalence of many psychiatric disorders (Cover et al., 2014). However, very little is known about even the most basic questions regarding the rewarding properties of same-sex social interactions, particularly in females. Thus, there is a critical gap in our knowledge about the basic behavioral characteristics and properties underlying social reward and the neurobiological mechanisms encoding social reward, especially in females. Thus, for this dissertation we set out to methodologically investigate potential sex differences in the behavioral characteristics and neurobiological mechanisms underlying social reward.

Syrian hamsters were used to investigate these questions because they are a species particularly well-suited for studies investigating sex differences in social behavior and the preclinical study of behaviors that underlie psychiatric health and illness (Terranova et al., 2016). For example, like primates, but unlike other common laboratory rodents, both males and females display highly robust dominant-subordinate relationships (Drickamer & Vandenbergh, 1973, Drickamer et al., 1973). Thus, any sex differences in the rewarding properties of social interactions are likely due to differences in the neural mechanisms encoding social reward and

not due to sex differences in the types or quality of social behavior. Furthermore, Syrian hamsters were used because hamsters have been successfully employed in prior studies of social motivation (Ferris et al., 2984, Solomon et al., 2007, Morrison et al., 2014, Song et al., 2014, Gray et al., 2015) and social reward (Meisel & Joppa, 1994, Gil et al., 2013, Song et al., 2016).

In the first set of experiments (Chapter 2) we validate a novel Operant Social Preference (OSP) task (operant instrument), which is less dependent on memory than the classical conditioned place preference test (classical conditioning), for a more detailed and direct assessment of the rewarding and motivating properties of same-sex social interactions. In the second set of experiments (Chapter 3) we investigate the effects of the duration of social interaction "social dose" and behavioral cost on the reinforcing properties of social interactions. Furthermore, we examine the effects of OTRs in the VTA on the reinforcing properties of social interactions. Finally, in a third set of experiments (Chapter 4) we investigate sex differences in social reward and sex differences in the oxytocin system's modulation of social reward. More specifically, we investigate the role of OT neurons in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) and OTRs in the VTA on social reward in males and females. Collectively, we test the overarching hypothesis that there is an inverted U-shaped relationship between duration of social interaction and social reward that is mediated by OT in both males and females, and that females are shifted to the left of the curve in regards to the rewarding effects of social interactions. Understanding the sex differences in the mechanisms of social reward is particularly important clinically because deficits in social reward are linked with a variety of psychiatric disorders (McGregor & Bowen, 2012, Foulkes et al., 2015), many of which are sex-dependent in terms of prevalence and predispositions, e.g. autism spectrum disorder (Young & Pfaff, 2014).

1.2 Background

Note: parts of this background have been published with contributions from co-authors: James K. Rilling, Kyle J. Frantz, H. Elliott Albers and anonymous reviewers

(Borland et al. 2019a)

1.2.1 Sex Differences in the Brain

The existence of sex differences in the brain was first recognized less than 60 years ago (Phoenix et al., 1959), and much remains to be learned about the physiological mechanisms and functional significance of these sex differences (De Vries, 2004; McCarthy et al., 2015). Sex differences in the brain not only underlie sex differences in reproductive behavior but also play an important role in the expression of social behaviors such as aggression (Bales and Carter, 2003; Terranova et al., 2016), social play (Veenema et al., 2013; Bredewold et al., 2014), and social communication (Telgkamp et al., 2007; Albers, 2012). A major focus of the study of sex differences in the brain has been neurochemical signaling, revealing sex differences in the distributions of both signaling molecules and their receptors (Cosgrove et al., 2007; Panzica and Melcangi, 2016). The first, and perhaps the most pervasive, sex difference identified in the mammalian brain to date is the distribution of arginine-vasopressin (AVP), a peptide of the arginine-vasopressin/oxytocin family of nonapeptides (i.e., peptides composed of nine amino acids). For example, AVP immunoreactivity is greater in cell bodies in the bed nucleus of the stria terminalis and medial amygdala in male rats than in females (De Vries et al., 1981). Although the functional significance of this sex difference in AVP immunoreactivity is not fully understood, sex differences in the AVP system do play an important role in regulating sex differences in at least some social behaviors (Albers, 2015; Terranova et al., 2017). For example, AVP regulates aggression by acting within the hypothalamus in opposite ways in males

and females; AVP stimulates aggression in males and inhibits aggression in females (Ferris et al., 1997; Caldwell and Albers, 2004; Gutzler et al., 2010; Terranova et al., 2016). Another member of this family of peptides, oxytocin (OT), also plays a major role in the regulation of social behavior, especially those behaviors related to social bonding in particular (Carter et al., 2008; Caldwell, 2017) and social reward in general, e.g. parental behavior, pair bonding, and trust (Young and Wang, 2004b; Groppe et al., 2013). Given the likely importance of OT for regulating social reward in males and females, this dissertation explores the literature on sex differences in the neurobehavioral mechanisms mediating social reward, with a focus on the role of OT. We also put forth a new hypothesis that an inverted-U shape describes the relationship between social stimulation and social reward, perhaps mediated by OT.

1.2.2 OT and AVP signaling and Social Behavior

The AVP/OT family of peptides is evolutionarily ancient, and their structures are remarkably similar (Acher and Chauvet, 1995). In mammals, the structure of the two most important members of this peptide family, OT and AVP differ in only two amino acids. The canonical receptors for these peptides include OTR for OT, and V1aR, V1bR and V2R for AVP, with V1aR serving as the dominant AVP receptor in the brain. As in the similarity of the structure of these peptides, these receptors share at least 25% of their primary structure (Gimpl and Fahrenholz, 2001). As a result, it is not surprising that substantial cross-talk has been identified, through which OT activates AVP receptors and vice versa (Schorscher-Petcu et al., 2010; Song et al., 2014; Song et al., 2016b). Therefore, the functions of the OT system cannot be entirely divorced from the functions of the AVP system. While exogenously administered OT activates AVP receptors, and exogenously administered AVP activates OTRs in many cases, the extent to which endogenously released OT or AVP activate each other's canonical receptors is

not known (Song and Albers, 2017). These complexities in cross-talk must be considered and explored in the context of sex differences as well. Although the focus of this review will be on OTRs, a full understanding of the effects of OT on social behavior and reward in males and females will also require studies of potential sex differences in the activation of AVP receptors by OT.

OTRs are members of an evolutionarily ancient superfamily of G protein-coupled receptors composed of seven putative transmembrane domains (Gimpl and Fahrenholz, 2001; Hazell et al., 2012). OTRs are coupled to G_q and G_i protein complexes, and their activation can result in a range of complex cellular effects that remain to be fully delineated (van den Burg and Neumann, 2011; Busnelli and Chini, 2017). Interestingly, however, the coupling of OTRs to different G proteins can result in diverse, even opposing, effects within the cell (Gravati et al., 2010). Moreover, the concentration of OT appears to determine the coupling of OTRs to different subtypes of G proteins (Busnelli et al., 2012). Therefore, the concentration-dependent behavioral effects of OT might be the result of concentration-dependent effects on the coupling of OTRs to different G protein subtypes (see below). It will be important to consider that diverse types of OTR coupling may occur in neurons of differing phenotypes, and that these differences are likely to have functional consequences in the control of social behavior. Indeed, there is evidence of sex differences in the strength of coupling of neuropeptide receptors to their G-proteins (Bangasser et al., 2010).

1.2.3 Central distribution of OT and AVP

Because of the potential for AVP, as well as OT, to activate OTRs it is important to consider sex differences in the levels of AVP and OT expressed within specific brain regions that may contribute to the regulation of social behavior. The distribution of OT- and AVP-containing

neurons have been reviewed recently (Dumais and Veenema, 2016; Caldwell, 2018), so they are summarized only briefly here. High levels of OT and AVP are expressed in magnocellular neurons of the hypothalamus (e.g., paraventricular nucleus (PVN), supraoptic nucleus (SON) and nucleus circularis). Both OT and AVP are also produced by smaller populations of parvocellular neurons in several brain regions, e.g., amygdala. Sex differences in the levels of OT are described for just a few brain regions to date, although the findings are not consistent across species. For example, higher levels of OT immunoreactivity have been reported in neuronal cell bodies in the PVN and SON of females compared to males in several species such as mice (Haussler et al., 1990; Qiao et al., 2014), but no sex differences in OT immunoreactivity in neurons of these regions has been reported in several other species, including humans and rats (Caffe et al., 1989; Wang et al., 1996; Wang et al., 1997; Ishunina and Swaab, 1999; Rosen et al., 2008). As noted above, major sex differences in the number of AVP-containing cell bodies have been reported for the amygdala and bed nucleus of the stria terminalis and in their projections in rats (De Vries et al., 1981; van Leeuwen et al., 1985). Subsequent studies detailed similar sex differences in AVP levels in the extended amygdala, PVN, and SON of several other species as well (Delville et al., 1994; Wang, 1995; Wang et al., 1997; Ishunina and Swaab, 1999; Qiao et al., 2014). Although these sex differences are not uniformly present in all species (Caffe et al., 1989; Albers et al., 1991; Steinman et al., 2015), it is noteworthy that when sex differences were observed in the regions producing the highest levels of OT and AVP (i.e., the PVN and SON), higher levels were seen in females than in males.

The factors regulating the release of OT and AVP are not fully understood, but likely involve both synaptic and non-synaptic mechanisms (Ross and Young, 2009; Knobloch and Grinevich, 2014; Albers, 2015; Chini et al., 2017). OT and AVP have been identified in dense

core vesicles in synaptic regions of neurons in a variety of brain sites, and these peptides can be released in a calcium-dependent manner (Buijs and Swaab, 1979; Buijs and Van Heerikhuize, 1982; Buijs, 1983). Thus, local activation of AVP/OT receptors could be produced by the synaptic release of these peptides, or possibly by their release from axons of passage. In contrast, when AVP/OT are released from non-synaptic regions of neurons (e.g., dendrites), the peptides are thought to spread far more widely than following synaptic release, although the distance of spread is not clear (Engelmann et al., 2000; Leng and Ludwig, 2008; Chini et al., 2017). Non-synaptic release of AVP/OT has been most extensively studied in magnocellular neurons, but non-synaptic release can also occur in parvocellular neurons (Castel et al., 1996). Importantly, social interactions result in significant levels of activity in OT-containing magnocellular neurons of the PVN and SON in males and females. Because these magnocellular neurons produce some of the largest amounts of OT in the brain, their activation by social interactions likely results in substantial elevations of OT throughout the brain.

1.2.4 Central distribution of OTRs

Sex differences in the distribution and number of receptors for OT have been observed within specific brain regions, although these sex differences, like those for the expression levels of OT and AVP, are not entirely consistent across species (Dumais and Veenema, 2016; Caldwell, 2018). The distribution of OTR binding tends to be greater in males compared to females in forebrain regions where sex differences have been reported in a number of rodent species including rats, mice, and prairie vole males, e.g., the nucleus accumbens (NAc) (Dumais et al., 2013; Dumais and Veenema, 2016; Caldwell, 2018; Donovan et al., 2018; Guoynes et al., 2018). However, these male/female differences are not always consistent. For example, OTR binding in the CA1 region of the hippocampus is higher in males than females in rats (Dumais et

al., 2013) but lower in males than females in mice (Insel et al., 1991; Dumais et al., 2013). Gonadal hormones also influence OTR binding in specific brain regions, in a species-specific manner (De Kloet et al., 1986; Johnson et al., 1989; Tribollet et al., 1990; Bale et al., 1995). For example, estrogen selectively influences OTR binding in the anterior olfactory nucleus in prairie voles and in the ventromedial hypothalamus in rats (Witt et al., 1991; Bale and Dorsa, 1995). Of significant note, OTRs are distributed in key structures of what has been called the social decision-making network that includes structures important for social behavior and reward (O'Connell and Hofmann, 2011; Caldwell and Albers, 2016).

1.2.5 OT in the mesolimbic dopamine system

There is strong support for the role of OT in regulating social reward by its actions in the mesolimbic dopamine system. This system is critical for the rewarding properties of many stimuli, including food (Wei et al., 2016), water (Mikhailova et al., 2016), drugs of abuse, and various social interactions (Grotewold et al., 2014; Gunaydin et al., 2014; Kummer et al., 2015). The primary pathway of the mesolimbic circuitry is dopamine (DA) containing neurons in the ventral tegmental area (VTA) that project to the NAc (Bjorklund and Dunnett, 2007; Ikemoto, 2007; Beier et al., 2015). Social interactions can increase neuronal activity in both the VTA and NAc in male hamsters, rats and mice (El Rawas et al., 2012; Gil et al., 2013; Kummer et al., 2015), and selective activation of this DA projection increases social motivation in mice (Gunaydin et al., 2014). Indeed, the activation of OTRs in the mesolimbic circuit appears to be necessary for social interactions to be rewarding in male rodents. For example, activation of OTRs in the NAc appears necessary for social reward in male mice (Dolen et al., 2013), and activation of OTRs in the VTA also appears necessary for social reward in male hamsters and mice (Song et al., 2016a; Hung et al., 2017). OT-containing projections innervate structures in

the mesolimbic DA system and play an important role in mediating the rewarding properties of a range of stimuli. OT is synthesized in several hypothalamic nuclei including the PVN and SON and OT containing fibers project from these sites to mesolimbic dopamine structures such as the NAc and VTA (Melis et al., 2007; Ross et al., 2009; Knobloch et al., 2012) (Figure 2A). In the VTA, OTRs are found on DA containing neurons that project to the NAc, as well as to other structures (Peris et al., 2017). These fibers appear to stimulate DA activity, as exogenous OT injected into the caudal VTA leads to DA efflux in the NAc (Melis et al., 2007). Finally, OT-containing neurons in the PVN projecting to the VTA are necessary for social reward in male mice (Hung et al., 2017).

1.2.6 Sex differences in mesolimbic dopamine signaling

The structure and function of the mesolimbic system are sexually differentiated, with many reports suggesting higher basal and stimulated activity in the system among females compared with males (for a review see (Gillies et al., 2014)). For example, morphological sex differences include more cell bodies containing DA and greater volume in the VTA of female rats compared to males (McArthur et al., 2007), as well as sex differences in the projections of DA-containing VTA neurons (Kritzer and Creutz, 2008). On a functional level, the same degree of electrical stimulation of medial forebrain bundle fibers leads to greater DA efflux in the NAc of female rats, compared to males (Walker et al., 2000). Moreover, basal extracellular levels of DA in the NAc are higher in female rats compared to males (Virdee et al. 2014), and females display a faster rate of DA uptake and release (Walker et al. 2006). When stimulated by drugs that block or reverse the DA transporter, higher levels of extracellular DA are seen in females than males (Walker et al., 2006). Notably, however, an interesting dichotomy was reported in one study, such that amphetamine increased extracellular DA in male rats while decreasing it in

females (Virdee et al., 2014). This increased sensitivity among females to psychostimulant drug effects is also associated with greater immediate early gene expression across the middle and caudal striatum (Castner and Becker, 1996). Postsynaptically in the NAc, the balance between the D1 and D2 families of receptors may vary across the sexes and across the estrous cycle in females (Becker and Hu, 2008). Similar sex differences extend to humans (Mozley et al., 2001); e.g. women have a higher synaptic concentration of DA in the striatum than men (Laakso et al., 2002), as well as a stronger ventral striatum response to prosocial decisions (Soutschek, 2017). At least some of these sex differences are mediated by estrogen, as variations across estrous and menstrual cycles suggest increased activation of DA pathways when estrogen levels are high or rising, while ovariectomy attenuates sex differences (Becker and Cha, 1989; White et al., 2002). On the other hand, gonadectomy has little effect in males (Robinson et al., 1981; Becker and Beer, 1986; Forgie and Stewart, 1994).

1.2.7 Sex differences in social reward

The opportunity for social interactions appears to compete effectively with other rewarding stimuli to determine behavioral outcomes, perhaps to a greater extent in females than males. For example, social stimuli might trigger greater DA release in females than males, as shown among control groups in a study of rats exposed to same-sex stimulus animals after a period of social isolation (Grotewold et al., 2014). Furthermore, in female rats, levels of DOPAC (a metabolite of DA) in the striatum were elevated during same-sex social interactions, while in males, no difference in DOPAC levels were observed (Weiss et al., 2015). In addition, social housing can reduce drug intake in operant conditioning models, compared to social isolation housing (Chauvet et al., 2009; Thiel et al., 2009; Raz and Berger, 2010; Bregolin et al., 2017), an effect that is more consistent in females than in males, and may be mediated by OT in

mesolimbic regions (Bozarth et al., 1989; Westenbroek et al., 2017). OT itself also decreases drug-related reward and reinforcement (Carson et al., 2013), with greater effects in females than males (Cox et al., 2013; Leong et al., 2016). The mechanisms underlying the sex differences in social reward are not known, although sex differences in gonadal hormones are likely to play a role, as they do in mediating sex differences in drug reward (Becker and Hu, 2008; Becker, 2016). In translation to the human condition, it appears clear that social support reduces drug use, ameliorates stress, and predicts better outcomes in the treatment of various disorders (Havassy et al., 1995; Dobkin et al., 2002), but sex differences in this arena are not well documented.

1.2.8 An inverted U hypothesis to interpret sex differences in OT effects

A sex-dependent inverted U function has been hypothesized for the relationship between brain OT levels and neural activity in human studies (Feng et al., 2015a) (see below). Here, we extend the inverted U hypothesis to explain various effects of OT on social behaviors in male and female subjects. OT administered peripherally or centrally appears to have some rewarding properties of its own in both male and female rodents. Rodents spend more time in the chambers associated with OT treatment, and will self-administer OT (Liberzon et al., 1997; Donhoffner et al., 2016). OT may mediate social reward by its actions in the mesolimbic DA system, especially in the VTA where activation of OTRs is essential for the rewarding properties of social interactions in male mice and hamsters (Song et al., 2016a; Hung et al., 2017; Borland, 2018). As noted above, the ability of OT to reduce responses to drug reward may be more robust in female rats than males (Cox et al. 2013).

Data sets from other rodent studies are also consistent with this inverted U relationship. In female mice, intracerebroventricular injections of OT induce a conditioned social preference

for female stimulus mice at lower concentrations, but this preference is lost at higher concentrations (Kent et al., 2013). Using intranasal administration of OT, sex differences in the rewarding properties of social interactions have also been studied (Kosaki and Watanabe, 2016). In female mice, pairing 12 μg intranasal OT with the presence of a same-sex stimulus mouse induced a preference over another same-sex stimulus mouse not paired with OT administration. Interestingly, when the concentration of OT was increased to 36 μg and the trials extended, the initial preference for the stimulus mouse was eliminated, and the stimulus mouse even appeared to became aversive. In contrast, in males, administration of 12 μg OT had no effect on the preference for a same-sex stimulus mouse. These data support the hypothesis that males are less sensitive to the reward-enhancing effects of OT, and that increasing concentrations of OT initially increase reward, but can subsequently lead to an aversive response at higher concentrations, at least in females.

1.2.9 OT and Social Reward- Human Studies

In humans, OT effects on social reward processing have been investigated by examining the effects of intranasal OT administration on behavior and brain function, as measured by functional magnetic resonance imaging (fMRI). While several studies have examined the effects of intranasal OT administration on social reward processing in either men or women (Weisman et al., 2012; Groppe et al., 2013; Scheele et al., 2013; Gregory et al., 2015; Scheele et al., 2016; Hecht et al., 2017; Li et al., 2017), few have compared men and women in the same study. In one double-blind, placebo-controlled, pharmaco-fMRI study, men and women were randomized to treatment with either 24 IU intranasal OT administration or placebo approximately 40 minutes before they received an fMRI scan, while playing a dyadic social interaction task involving positive (reciprocated cooperation) and negative (unreciprocated cooperation) social

interactions with same-sex partners. At baseline, women but not men showed bilateral activation in NAc in response to positive social interactions. Although caution must be exercised in inferring psychological processes from neural activations (i.e., reverse inference) (Poldrack, 2011), this result suggests that women may find positive social interactions with same-sex partners to be more rewarding or more salient than men do at baseline. However, pre-treatment with 24 IU intranasal OT significantly increased the NAc response to positive social interactions in men, along with the caudate and putamen. On the other hand, pre-treatment with 24 IU intranasal OT significantly decreased activation across many brain regions in women, including the putamen. A direct statistical comparison between men and women showed that intranasal OT increased the caudate and putamen response in men to a greater extent than in women. More specifically, the right caudate/putamen response to reciprocated cooperation was larger in women than in men at baseline, but intranasal OT treatment increased the response in men to the level of women in the placebo group. On the other hand, intranasal OT treatment decreased the response of women to the level of men in the placebo group (Feng et al, 2015). These results, coupled with evidence that women have higher baseline CSF OT levels (Altemus et al., 1999), support the hypothesis of an inverted-U shaped dose-response relationship between brain OT levels and neural response, whereby raising brain OT levels in men would augment the caudate/putamen response, moving them closer to the maximum. On the other hand, raising OT levels in women might displace them to the right of the response maximum, decreasing the brain response. This hypothesis is supported by studies demonstrating non-linear dose-response properties of intranasal OT administration in human males. 24 IU intranasal OT, as compared with placebo, reduced men's cortisol responses to physical stress, enhanced autobiographical memory retrieval, and promoted the retrieval of social affiliation memories associated with

more positive feelings (Cardoso et al., 2013; Cardoso et al., 2014). Pushing higher to the 48 IU dose no longer produced significant differences in stress response and actually reduced memory retrieval scores lower than the 24 IU dose for memory retrieval.

Collectively, these results show that 24 IU intranasal OT modulates the neural response to reciprocated cooperation very differently in men and women. In men, OT increases the response to social reward associated with reciprocated cooperation within areas involved in reward and salience, such as the caudate/putamen Importantly, these modulatory effects of OT were specific to interactions with human partners – similar effects were not found with computer partners. In women, intranasal OT administration actually decreased the neural response to positive social interactions (reciprocated cooperation) across brain regions (Feng et al, 2015). Further analysis at the genetic level indicated that effects of intranasal OT administration on the caudate nucleus response to reciprocated cooperation were driven by individuals with a GG genotype at OTR SNP rs53576 (Feng et al., 2015b). Finally, a subset of the participants in this study returned for a second session as part of a within-subject design to evaluate OT effects. While OT did not increase the striatal response to positive social interactions in men, it robustly decreased the VTA response to positive social interactions in women (Chen et al., 2017).

In addition to examining how intranasal OT administration modulated activation within individual brain areas, data from this same study were analyzed to determine whether intranasal OT administration modulated functional connectivity across a neural network that animal studies implicate in social behavior. Intranasal OT administration induced widespread increases in functional connectivity in response to positive social interactions among men and widespread decreases in functional connectivity in response to negative social interactions among women.

Regions known to receive mesolimbic DA projections such as the NAc and lateral septum were hubs for intranasal OT administration effects on functional connectivity, again consistent with the notion that 24 IU intranasal OT administration enhances social reward processing in men but not women (Rilling, 2018). It should be noted however that fMRI does not measure DA signaling directly, nor can it reveal if activations within areas like the NAc necessarily involve the mesolimbic DA system.

As mentioned above, same-sex social interactions are more rewarding for female than male hamsters, and the same dose of OT can significantly increase social reward in males while decreasing social reward in females. The human results described above closely parallel these findings in hamsters. Nevertheless, other evidence suggests that human sex-differences in intranasal OT administration effects on social reward processing may be context or relationship-specific. For example, one study showed that 24 IU intranasal OT administration enhanced the pleasantness of a romantic partners' touch and also increased neural responses to the partners' touch in the NAc and anterior cingulate cortex (Kreuder et al., 2017). In contrast to the above study, there was no evidence for sex differences in OT effects on the perceived pleasantness of partner touch, and OT effects on the NAc response were actually stronger among women than men. These effects of intranasal OT administration were specific to touch from an assumed romantic partner and did not generalize to touch from an unfamiliar person. In fact, intranasal OT administration actually decreased the NAc response to touch by a stranger. The same research team has shown that intranasal OT administration increases the perceived attractiveness of, and NAc response to, male romantic partners among women (Scheele et al., 2016). However, this effect was not observed when women viewed pictures of familiar men who were not their partner. Two other studies in women have shown intranasal OT

administration to increase VTA responses to pictures of infant and sexual images (Gregory et al., 2015), and to cues that predict presentation of friendly faces (Groppe et al., 2013). Thus, the sex differences in the effects of intranasal OT administration on social reward processing mentioned above, including decreased activation in social reward processing regions in women, may be specific to positive social interactions with same-sex and/or unfamiliar individuals. Alternatively, OT attenuation of social reward in women may only apply for social stimuli that are sufficiently rewarding at baseline (i.e., near the peak of the dose-response function). For less rewarding social stimuli on the ascending portion of the inverted U function, OT would instead increase social reward. Thus, for example, if a woman has habituated to her partner's touch, it may not be highly rewarding at baseline, but OT treatment may render it more so. In summary, as is the case in most areas of social neuroscience, the mechanisms underlying social reward have been investigated more extensively in males than in females. Thus, in this dissertation work we set out to systematically investigate the rewarding properties of social interactions in both male and female hamsters and describe the potential sex differences in the role of OT in regulating social reward.

2 CHAPTER TWO: A NOVEL OPERANT TASK TO ASSESS SOCIAL REWARD AND MOTIVATION IN RODENTS

Note: this work has been published with contributions from co-authors: Kyle J. Frantz, Lauren M. Aiani, Kymberly N. Grantham, Eric Song, H. Elliott Albers and anonymous reviewers

(Borland et al. 2017)

2.1 Abstract

Social reward plays a critical role in the development of beneficial social relationships, and disorders of the mechanisms controlling social reward are involved in the etiology of many psychiatric diseases. We present a novel operant social preference task to quantify social reward in rodents using an apparatus with three chambers separated by one-way vertical-swing doors. The experimental animal is placed in the larger chamber while the two smaller chambers either remain empty or contain a stimulus animal or other potential reward stimulus. Adding weights to the door can alter effort required for rewards. Hamsters (Mesocricetus auratus) entered the chamber containing a stimulus hamster significantly more frequently than an empty chamber. When the reinforcing effects of social interactions were compared to food reward under progressive cost requirements, the reinforcing effects of social interaction and sunflower seeds were similar. Progressively increasing the door weight decreased number of entries, but increased time spent attempting to open the doors. The quantification of the rewarding properties of social interactions has almost exclusively used the conditioned place preference (CPP) paradigm. Although robust and reliable, CPP includes a memory component, because it relies on the association of place with the social interaction while the operant task presented here does not. This task allows for detailed and direct assessment of social and non-social rewards

that may serve as effective behavioral reinforcers in this operant conditioning model, and it can be used to investigate the neural mechanisms regulating motivation.

2.2 Introduction

The rewarding properties of social interactions are critical for the expression of adaptive social behaviors, including the development of social relationships in most species (Darwin, 1859; Thorndike, 1905; Skinner, 1938; Lorenz and Leyhausen, 1973; Oliveria et al., 1998; Pettinger et al., 2011; Pusey and Packer, 1997; Krach et al., 2010). In humans, deficits in the rewarding properties of social stimuli likely contribute to many psychiatric disorders (Dichter et al., 2012; Stravropoulos and Carver, 2013; Foulkes et al., 2015; Novacek et al., 2016). The basic neural mechanisms regulating social reward have been investigated in rodent models almost exclusively with the conditioned place preference (CPP) paradigm (Calcagnetti and Schechter, 1992; Meisel and Joppa, 1994; Peartree et al., 2012; Dolen et al., 2013; Gil et al., 2013; Song et al., 2016). Although robust and reliable, CPP includes a memory component, because it relies on the association of place with the social interaction (Trezza et al., 2011). In other words, reward value is operationalized as time spent in the area associated with the memory of the rewarding stimulus, even though the presumed reward is not present at the time of testing. Here, we present a novel operant social preference (OSP) task that more directly quantifies social reward; as an operant task that tests the reinforcing effects of a visible social stimulus, it does not rely on memory.

Other operant conditioning tasks investigate the rewarding properties of opportunities to interact with a conspecific using lever-pressing or nose-pokes (Martin and Iceberg, 2015; Achterberg et al., 2016). For the first time here, movement through one-way vertical-swing doors is the operandum used to assess motivation to interact with a conspecific (Olsson and Keeling, 2002; Wirth et al., 2003; Seaman et al., 2006; Tilly et al., 2010). This new operant task allows investigation of whether social interaction reinforces entries into a separate chamber. As

with other operant tasks, if placing a stimulus in the chamber increases chamber entry behavior (the operandum), then that stimulus is likely to be serving as positive reinforcement with some reward value. This task is less dependent on memory than other tasks, because holes in the doors allow visual, auditory and olfactory stimuli to be detected throughout the test session. Reward value can be quantified by directly measuring number of rewards "consumed" and allowing subjects the choice to access reward or not. In addition, progressively increasing the weight of the door allows assessment of reward value via its relationship with energy expenditure (Beeler et al., 2012). Syrian hamsters were used to validate this novel task, because hamsters have been successfully employed in studies of social motivation (Ferris et al., 1984; Solomon et al., 2007; Morrison et al., 2014; Song et al., 2014; Gray et al., 2015) and social reward (Meisel and Joppa, 1994; Gil et al., 2013; Song et al., 2016a). They also provide an excellent model for pre-clinical studies of psychiatric disorders (Terranova et al., 2016).

To validate this novel task, we tested whether same-sex social interactions would reinforce the acquisition of an operant task, followed by testing its extinction in the absence of the social stimulus, and its reinstatement by re-introducing the social stimulus (Suomi et al., 1971, Phillips and Fibiger, 1990; Ranaldi and Roberts, 1996; Sapolsky, 2015). We also compared the reinforcing effects of social interaction with a more conventional food reward, sunflower seeds, in both acquisition conditions and under progressive increases in door weights (Rickard et al., 2009). If this novel OSP task is a valid measure of reward, then the presentation of rewarding stimuli should decrease latency and increase frequency of entries into chambers containing rewarding stimuli compared to empty chambers. Further we examined whether social and food rewards have similar reinforcing properties.

2.3 Materials and Methods

Subjects

Male Syrian hamsters (n=34, 120-140 g) arrived from Charles River Laboratory (Wilmington, MA) at 11 weeks old and were housed singly in a humidity and temperature controlled (22^{o}C) vivarium. All animals were housed in solid-bottom Plexiglas cages (43 x 22 x 20 cm) containing corncob bedding and cotton nesting material (Neslets; Ancare, Bellmore, NY) in a reverse light-dark (LD) cycle (14L:10D; lights off at 13:00). Food and water were available *ad libitum*. Hamsters acclimated for 4 weeks before experiments. Hamsters were weighed just prior to their first behavioral test and again at the end of their last behavior test. All behavioral tests were performed under red light during the first 3 hours of the dark phase of the LD cycle. All procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and approved by the Georgia State University Institutional Animal Care and Use Committee.

Operant Social Preference Apparatus

The OSP apparatus was constructed of clear acrylic (Custom Plastics, Decatur, GA, USA) (Figure 2.1). The apparatus consisted of three chambers: a main chamber (50.8 x 33 x 30.5 cm, *l x w x h*) and two smaller adjacent chambers (25.4 x 17.8 x 30.5 cm, *l x w x h*). Each small chamber is separated from the main chamber by a one-way vertical-swing door (10.2 x 7.6 cm, *l x h*); smaller chambers can only be accessed from main chamber. Chamber doors were brushed with steel wool to achieve coarse texture, distinct from rest of apparatus, and doors were perforated by circular holes to allow airflow. Buckets that served to hold weights [85g (3oz), 113g (4oz) and 170g (6oz)] were attached to each door on the small chamber side.

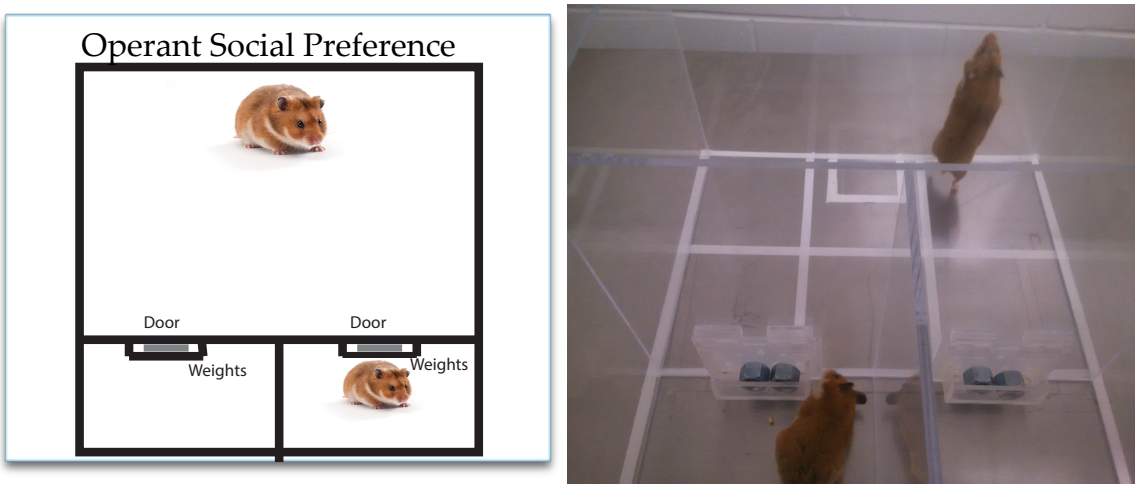


Figure 2.1: Operant Social Preference Apparatus: schematic (left) and vertical view (right). The main chamber contains an experimental subject and a stimulus hamster occupies one of the side chambers, separated by the vertical-swing door. The door allows visual, auditory and olfactory cues to enter the small chambers through the open top and holes in the door. Drop zone rectangular box is to the immediate left of subject hamster.

Operant Social Preference Conditioning

Operant conditioning sessions began with hamsters placed in a designated drop zone (10.2 x 7.6 cm, *l x h*) against the far wall of the main chamber in the OSP apparatus, equidistant from both small chambers. A smaller (100-120g) non-aggressive (group housed), same-sex stimulus hamster was confined to either the left or right smaller chamber. Assignment of the stimulus hamsters to the right or left chamber was counter-balanced across experimental subjects. Subjects never interacted with the same stimulus hamster across testing days: a new stimulus hamster was provided for each subject on each test day. Subjects were allowed to move throughout the apparatus, while stimulus hamsters were confined to one of the small chambers. Twenty seconds after entry into either small chamber, the subject was returned to the drop zone

in the main chamber. An initial acquisition session lasted between 10-30 minutes; each subject was required to enter the chamber holding the stimulus animal at least 3 times. Time spent in the apparatus for controls without social interaction (both chambers empty) was yoked with subjects that were experiencing social interaction. All hamsters received at least two more acquisition sessions on two consecutive days. Additional sessions were conducted if criteria for acquisition were not met (at least 2 social entries for 2 consecutive days). No extra acquisition sessions were needed in these experiments. All test sessions except for the first acquisition session were 10 min in duration. Hamsters progressed from no weights in the door buckets during the first 2 days to 113g for the next 2 days during acquisition testing. Only subjects that met the acquisition criterion of least 2 social entries into the chamber holding the stimulus hamsters during the 10-min session for two consecutive days were included in subsequent experiments. Displays of submissive behavior in the presence of conspecifics were also grounds for exclusion (two in experiment 2).

Progressive Weights Schedule: To assess motivation under higher cost requirements, test sessions began under the same procedure as described above except door weights were progressively increased over consecutive days starting at 113g (4oz), 227g (8oz), 340g (12oz), 454g (16oz), 634g (22oz), 794g (28oz)].

Food Reinforcer: To compare reward-related behavior between social stimulus and food stimulus, 10-15 sunflower seeds were placed in one of the small chambers, rather than a stimulus hamster. Ten to fifteen unsalted shell-less seeds were maintained in the chamber throughout the test session. (Sunflower seeds were replenished to 10-15 after each entry, if consumed by a subject). Otherwise, all conditions were the same.

Behavior Scoring

All behavior tests were recorded and analyzed using the Noldus Observer system. A scorer blind to treatments and groups scored each videotape. In addition to chamber entries and latency to chamber entry, the following mutually exclusive behaviors were scored: duration of aggression, social investigation, submission (e.g. fleeing, avoidance), grooming and non-social behavior. The frequency of attacks was scored as a point event during displays of aggression, and frequency of flank marks was scored as a point event during non-social behavior. For operational definitions of these behaviors, please see (Drickamer et al., 1973; Gray et al., 2015). Flank marking was scored due to its strong link to dominance status and territoriality in rodents (Ferris et al., 1987; Albers and Rowland, 1989). Generally these behaviors were measured to enable description of how time was spent in the small chamber, ultimately aiding conclusions about their ability to reinforce the chamber entry behavior. Time spent pushing door open was measured by scoring duration in which subject hamster displaces either door from normal resting position. Locomotor activity was also scored: the OSP apparatus was subdivided into 6 equal-sized squares (16.9 x 16.5 cm) and the number of square entries was counted.

Experimental Design and Statistical Analysis:

Data were analyzed using SPSS software (SAS Institute, 1990) 23.0 for Windows. All data were examined to determine if the assumptions of parametric statistical tests were met. When assumptions were violated, data were square root transformed (number of chamber entries and grooming duration in exp. 1 and time spent pushing door open in exp. 2) or cube root transformed (frequency of flank marks in exp. 1). All tests were two-tailed, and results

considered statistically significant if $p \leq 0.05$. All data are presented as mean ± standard error of the mean.

Experiment 1: *Effects of Social Interaction on Chamber Preference.*

Male Syrian hamsters were assigned to either the social exposure group (n=8) or the no social exposure control group (n=8). In the social exposure group, hamsters were tested with a stimulus hamster in one of the small chambers for 7 consecutive days (acquisition training, social days: SD), followed by three days with both chambers empty (extinction, extinction days: ED), followed by a final day on which a stimulus hamster was reintroduced into one of the small chambers (reinstatement test). In the no social exposure control group, both small chambers were empty for all 11 sessions. Body weights of hamsters were counter-balanced between groups.

In Experiment 1, data were averaged over the last 3 acquisition days and the 3 extinction days. Mixed or repeated measures ANOVAs were performed to examine effects of treatment condition (between subjects: social versus no-social), chamber condition (within subjects: stimulus hamster versus empty), and training condition (within subjects: acquisition versus extinction, or separately over each of the 11 test days) on number of entries and latency to enter chambers, grooming duration, frequency of flank marks, aggression duration and duration of social investigation. Post-hoc comparisons were carried out as appropriate using paired and independent sample t-tests. Correlations were carried out to investigate potential relationships between social behaviors and number of social entries or social preference scores (number of entries into social chambers – number of entries into empty chambers) on test days 1, 4, 6, 7, and

11. To control for experiment-wise error, alpha levels were adjusted according to the Holm-Bonferroni method.

Experiment 2: Comparison of Effects of Social Interaction and Sunflower Seeds on Chamber Preference.

First in Experiment 2 male hamsters were assigned to either the social exposure group (n=10) in which one of the small chambers contained a stimulus hamsters, or a food exposure (sunflower seed) group (n=8) in which one of the chambers contained sunflower seeds. Acquisition of a preference for the chamber containing the stimulus hamster or sunflower seeds, over an empty chamber, was tested over 4 consecutive days. Second in Experiment 2, after stable acquisition of door entries for social or sunflower preference at 0g and 113g, door weights were increased daily from 227g, to 340g, 454g, 634g and 794g. On the final day of testing, door weights were returned to 113g. Two social condition subjects were excluded for not meeting acquisition criteria, or displaying submissive behavior when in the presence of a conspecific. Body weights of hamsters were counter-balanced between groups.

For experiment 2, data for 0g and 113g were averaged across multiple test days. Mixed measures ANOVAs were used to examine effects of treatment condition (between-subjects: social versus sunflower), chamber condition (within subjects: stimulus versus empty), and acquisition day (within-subjects: days 1-4) or weights (within-subjects: PWs 113g-794g) on number of entries and latency to enter chambers, social or sunflower preference scores and time spent pushing doors open. Post hoc comparisons were carried out using paired and independent sample t-tests.

2.4 Results

Experiment 1: *Effects of Social Interaction on Chamber Preference.*

To determine whether social interactions were rewarding based on their ability to reinforce chamber entry behavior, the number of entries into the chambers was analyzed in the social versus no-social group by averaging the last 3 acquisition and 3 extinction days and factoring them into a 2 x 2 mixed measures ANOVA. There was a main effect of treatment (social exposure versus no-social exposure: $p<0.001$, $F(1,14) = 117.830$) on entries into chambers. There was also an effect of training condition (acquisition versus extinction: $p=0.001$, $F(1,14) = 15.653$), and an interaction of treatment group and training condition ($p=0.001$, $F(1,14) = 19.635$) on entries into chambers (Figure 2.2a inset). Fewer entries into chambers were observed during extinction training than acquisition training for social exposure ($p=0.003$, $t(7)=4.350$), but not for the no-social exposure treatment groups ($p=0.402$, $t(7)=-0.585$). A 2 x 11 mixed measures ANOVA on entries into chambers on individual sessions revealed a main effect of social treatment ($p<0.001$, $F(1,14) = 103.447$), an effect of test session (11 test days: $p<0.001$, $F(10,140) = 3.578$), and a significant interaction ($p<0.001$, $F(10,140) = 3.958$; Figure 2a) on the number of entries into chambers. Independent sample t-tests with Holm-Bonferroni corrections revealed that social exposure increased the number of chamber entries on social acquisition days 2 ($p=0.006$), 3 ($p=0.009$), 4 ($p=0.001$), 5 ($p<0.001$), 6 ($p<0.001$), 7 ($p=0.001$), extinction days 1 ($p<0.001$), 2 ($p=0.005$) and social reinstatement day ($p<0.001$), but not extinction day 3 ($p=0.161$), compared to chamber entries in the no-social exposure treatment group.

With regard to latency to enter the first (or stimulus) chamber, a main effect of treatment group ($p=0.002$, $F(1,14) = 13.521$), test session ($p<0.001$, $F(10,140) = 11.395$) and interaction of

treatment group and test session ($p<0.001$, $F(10,140) = 5.940$) were all significant (Figure 2.2b). Social exposure decreases latency to enter chambers. The latency to enter chambers also decreased from the first test session to all subsequent test sessions ($p<0.001$). Independent t-tests revealed that social exposure decreased the latency to enter chambers on social acquisition days 5 ($p=0.003$, $t(14)=-3.894$), 6 ($p=0.003$, $t(14)=-3.763$), 7 ($p=0.006$, $t(14)=-3.274$), and the second extinction day ($p=0.001$, $t(14)=-3.958$), compared to no-social exposure group (Figure 2.2b).

To determine the effect of social interaction on chamber preference in the social exposure treatment group only, the number of entries into the chamber containing a stimulus hamster versus the empty chamber was averaged for the last 3 acquisition and 3 extinction days. A 2 x 2 repeated measures ANOVA revealed a main effect of within-subjects chamber stimulus (social vs. empty: $p<0.001$, $F(1,7) = 65.790$), a main effect of training condition ($p<0.001$, $F(1,7) = 38.769$), and an interaction ($p=0.021$, $F(1,7) = 8.773$; Figure 2.2c inset). Paired t-tests revealed that there were more entries into chambers containing stimulus hamsters compared to empty chambers during acquisition ($p<0.001$, $t(7)=6.440$) and extinction training ($p=0.003$, $t(7)=4.338$). Paired t-tests also revealed that extinction training decreased entries into chambers containing stimulus hamsters ($p=0.003$, $t(7)=4.350$), but not empty chambers ($p=0.577$, $t(7)=0.577$). A 2 x 11 repeated measures ANOVA revealed that there was a main effect of within-subjects chamber stimulus ($p<0.001$, $F(1,7) = 59.316$) and a main effect of test session ($p=0.007$, $F(10,70) = 2.723$) on number of entries into chambers for social exposure treatment group. There was no interaction of test session and within-subjects chamber stimulus on number of entries into chambers ($p=0.466$, $F(10,70) = 0.984$). There were fewer entries into chambers on the third extinction day compared to all other test days ($p<0.05$), except for the second and third acquisition days and second extinction day ($p>0.05$; Figure 2.2c).

With regard to latency to enter the stimulus chamber, a main effect of within-subjects chamber stimulus (social versus empty: $p=0.004$, $F(1,7) = 18.140$) was revealed in a 2 x 11 repeated measures ANOVA, with a shorter latency to enter chambers containing a stimulus hamster versus empty chambers (Figure 2.2d). There was also a main effect of test session ($p<0.001$, $F(10,70) = 29.818$) on the latency to enter chambers in social exposure treatment group, such that the latency was longer on the first day compared with all other days ($p<0.001$).

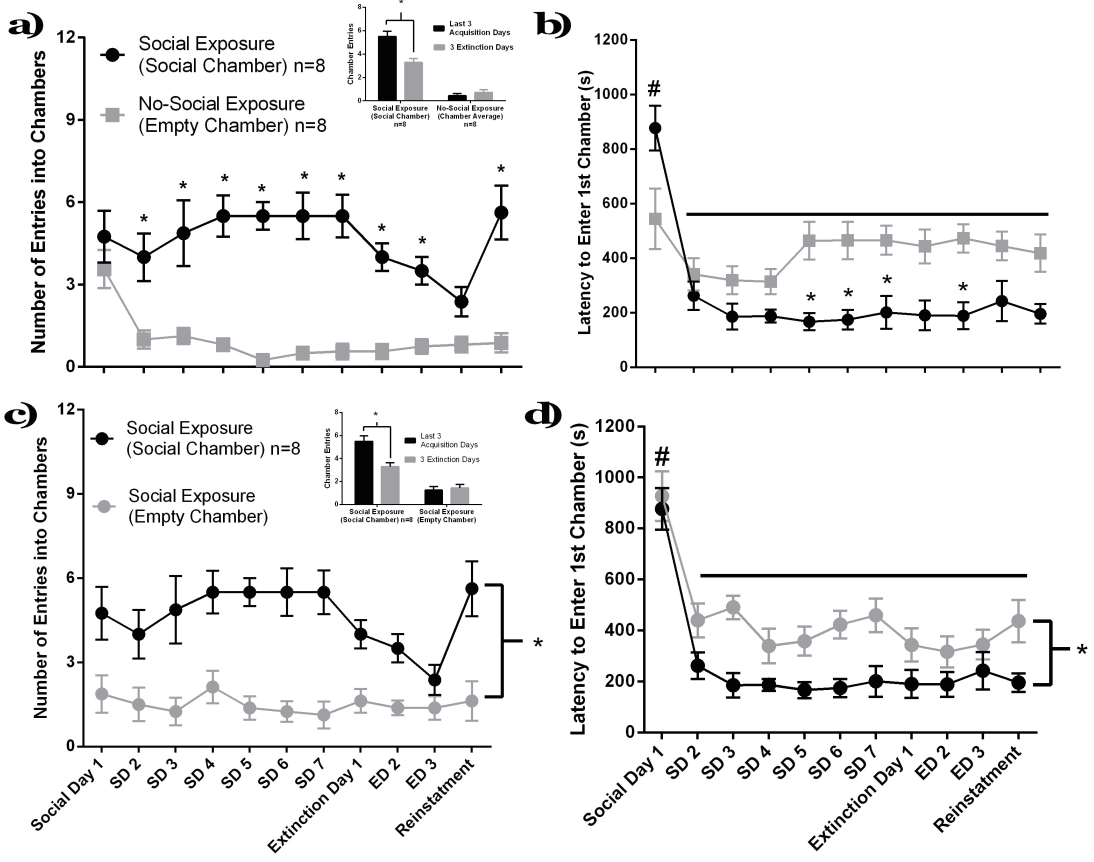


Figure 2.2: Comparison of the preference for chambers containing a stimulus hamster versus an empty chamber.

Panel a: Comparison between the group that had stimulus hamsters present in one chamber (social exposure) and the group that had only empty chambers (no-social exposure group) on

chamber entries. Social exposure results in more chamber entries than no-social exposure group on all days except first day of testing and third extinction day (* indicates significant difference between groups, $p<0.05$). Inset is comparing averages of last 3 acquisition days and 3 extinction days for each group. Removal of social stimulus decreases chamber entries in social exposure group. Panel b: Shorter latency to enter the chambers in the social exposure group developed to significant difference by day 5 compared to the latency to enter the chambers in the no-social control group. There was a shorter latency to enter the chambers from the first session compared to all subsequent sessions independent of treatment group (# indicates significant difference compared to all other test sessions). Panel c: In the social exposure group hamsters preferred the social chamber over the empty chamber (* indicates significant difference between chambers). Inset is comparing averages of last 3 acquisition days and 3 extinction days for each within-subjects chamber stimulus. Removal of social stimulus decreases entries in chambers containing a stimulus hamster, but not entries into empty chambers. Panel d: Similarly, the social exposure group showed shorter latency to enter the social chambers compared to empty chambers. There was a shorter latency to enter the chambers from the first session compared to all subsequent sessions independent of treatment group (# indicates significant difference compared to all other test sessions).

For scored social behaviors there was no main effect of treatment group ($p=0.090$, $F(1,14) = 3.313$) on number of flank marks as revealed by a 2 x 11 mixed measures ANOVA nor significant interaction ($p=0.344$, $F(10,140) = 1.138$; Figure 2.3a). There was a main effect of test session ($p=0.033$, $F(10,140) = 3.248$), with more flank marking on day 1 compared to days 8 ($p=0.020$), 10 ($p=0.036$) and 11 ($p=0.020$). For grooming, there was a main effect of treatment

(p=0.020, F(1,14) = 6.841) and an interaction of treatment condition and test session (p=0.020, F(10,140) = 2.217), but no main effect of test session (p=0.441, F(10,140) = 1.006; Figure 2.3b). Independent t-tests revealed more grooming in the no-social exposure group on days 4 (p=0.034), 5 (p=0.009), 6 (p=0.040), 8 (p=0.012) and 11 (p=0.019), compared to the social exposure group (Figure 3b). One-way ANOVAs revealed that neither the duration of social investigation (p=0.911, F(7,49) = 0.158; Figure 2.3c) nor the duration of aggression (p=0.355, F(7,49) = 1.142; Figure 2.3d), differed over the 8 days of social interaction testing in the social exposure treatment group.

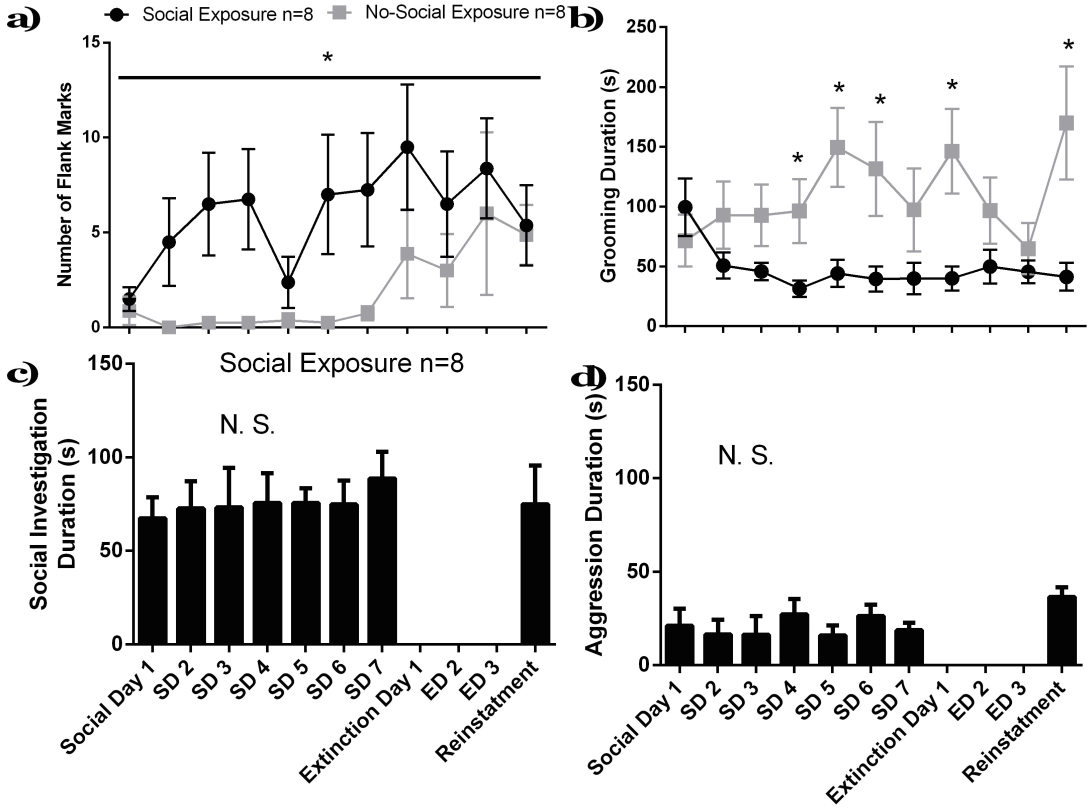


Figure 2.3: Effect of social exposure (one chamber containing a stimulus hamster) vs. no-social exposure (both chambers empty) on the number of flank marks expressed and the duration of grooming.

Panel a: Number of flank marks increases over the days of testing (* indicates main effect of test session). Panel b: More grooming was observed in no-social exposure group compared to social exposure group on test days 4, 5, 6, extinction test day 1 and reinstatement test day (* indicates difference in treatment group). Panel c: No significant difference in the duration of social investigation across all social test days in the social exposure group. Panel d: No significant difference in the duration of aggression across all social testing days in social exposure group.

Neither the number of entries into the chambers containing stimulus hamsters, nor social preference score (entries into social chamber – entries into empty chamber) correlated with the duration of aggression, duration of grooming, nor the number of flank marks on test days 1, 4, 6, 7 and 11 ($p>0.05$; data not shown). There was a trend for the duration of aggression to be positively correlated with number of entries into chamber containing stimulus hamsters on day 1 ($p=0.056$, $r=0.694$). However, as expected, the number of entries into chambers containing stimulus hamsters and social preference scores were correlated with the duration of social investigation on test sessions 1, 4, 6, 7 and 11 ($p<0.05$).

Experiment 2: Comparison of the Rewarding Properties of Social Interactions and Sunflower Seeds.

To compare the rewarding properties of social interactions and sunflower seeds during acquisition, one group of hamsters was allowed access to a chamber containing a stimulus hamster or an empty chamber, while another group was allowed access to either a chamber containing sunflower seeds or an empty chamber. The placement of the stimulus hamster or sunflower seeds in either the left or right chamber was counter-balanced between subjects.

Number of entries into chambers containing either stimulus hamsters or sunflower seeds was greater than the number of entries into empty chambers ($p<0.001$, $F(1,14) = 48.744$) as revealed by a 2 x 2 x 4 mixed measures ANOVA. There was no main effect of test session on number of entries into chambers ($p=0.090$, $F(3,42) = 2.312$), nor were there between group differences on number of entries into the chambers (social versus sunflower seed condition: $p=0.890$, $F(1,14) = 0.020$; Figure 2.4a).

The latency to enter chambers containing a stimulus hamster or chamber containing sunflower seeds was shorter than the latency to enter empty chambers ($p<0.001$, $F(1,10) = 28.338$) as revealed by a 2 x 2 x 4 mixed measures ANOVA (Figure 2.4b). There was a main effect of test session on the latency to enter chambers ($p<0.001$, $F(3,30) = 20.935$), such that that the latency to enter chambers decreased after the first day in comparison with days 2, 3 and 4 ($p<0.002$). There were no between group differences on the latency to enter chambers ($p=0.859$, $F(1,10) = 0.033$).

There were no between group differences on social or sunflower seed preference score ($p=0.445$, $F(1,14) = 0.616$), as revealed by a 2 x 4 mixed measures ANOVA (data not shown). There was also no main effect of test session on social or sunflower seed preference score ($p=0.489$, $F(3,42) = 0.822$).

To compare the rewarding properties of social interactions and sunflower seeds as the door weight was progressively increased; the same groups of hamsters from Experiment 2 were trained until the hamsters consistently chose the chamber containing the stimulus hamster over the empty chamber, or they chose the chamber containing sunflower seeds over the empty chamber, at 113g door weights. On subsequent trials the weights on both doors were progressively increased from 113g up to 794g over 5 consecutive days (227g-794g). On the final

day of the experiment, the door weight was returned to the standard 113g. Door weights had a significant effect on number of entries into chambers ($p<0.001$, $F(7,98) = 34.062$), as revealed by a 2 x 2 x 8 mixed measures ANOVA (Figure 4c). Specifically, as the weight of the door increased, the number of entries decreased ($p<0.05$). There were also significantly more ($p<0.001$, $F(1,14) = 53.937$) entries into chambers containing stimulus hamsters or sunflower seeds than into empty chambers. There was an interaction of weights and within-subjects chamber stimulus on number of entries into chambers ($p<0.001$, $F(7,98) = 13.267$). Subjects made more entries into chambers containing stimulus hamsters or sunflower seeds, compared to empty chambers at 0g, 113g, 227g, 340g, 454g, and reinstatement at 133g door weights as revealed by paired t-tests ($p<0.05$). There were no between group differences ($p=0.653$, $F(1,14) = 0.211$; Figure 2.4c) in number of entries into all chambers.

Door weights had an effect on the latency to enter chambers ($p<0.001$, $F(7,77) = 17.364$) as revealed by a 2 x 2 x 8 mixed measures ANOVA (Figure 4d). There was also a main effect of within-subjects chamber stimulus on the latency to enter chambers ($p<0.001$, $F(1,11) = 25.110$; Figure 2.4d), with a shorter latency to enter chambers containing a stimulus hamster or sunflower seeds than empty chambers. There was an interaction of within-subjects chamber stimulus and door weights on the latency to enter chambers ($p=0.050$, $F(7,77) = 2.128$). Subjects showed a shorter latency to enter chambers containing stimulus hamsters or sunflower seeds at 113g, 227g, 340g and reinstatement at 113g door weights, compared to empty chambers, as revealed by paired t-tests ($p<0.05$). There were no between group differences in the latency to enter chambers between social and sunflower seed conditions ($p=0.899$, $F(1,11) = 0.017$).

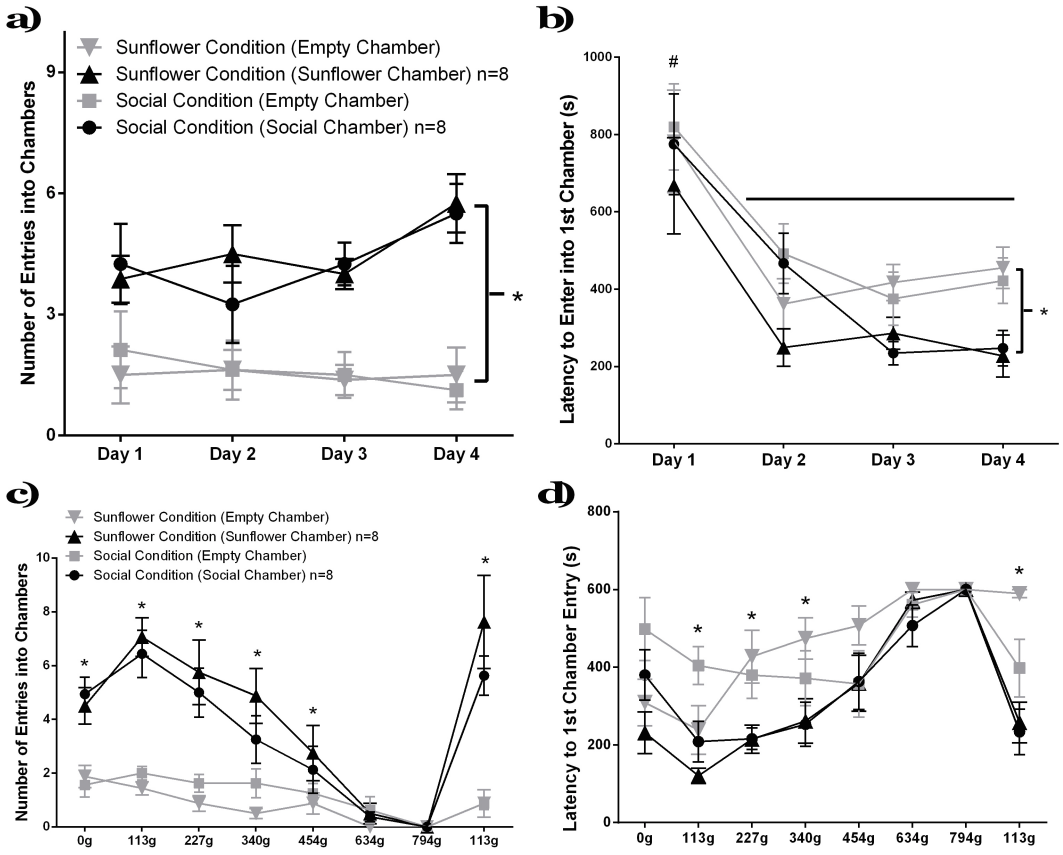


Figure 2.4: Chamber preferences of a group of hamsters that selected between a chamber containing a stimulus hamster or an empty chamber (Social Condition: n=8) or a group of hamsters that selected between a chamber containing sunflower seeds or an empty chamber (Sunflower Condition: n=8) during the acquisition of chamber preference and under progressively increasing weight of doors.

Panel a: More entries were observed into chambers containing stimulus hamsters or sunflower seeds compared to empty chambers (* indicates significant difference in within-subjects chamber stimulus). Panel b: Shorter latency to enter chambers containing stimulus hamsters or sunflower seeds compared to empty chambers. There was a longer latency to enter chambers on the first testing session compared to all subsequent testing sessions (# indicates significant difference compared to all other test sessions). Panel c: More entries were observed into chambers

containing stimulus hamsters or sunflower seeds compared to empty chambers at 0g, 113g, 227g, 340g, 454g and reinstatement of 113g door weights. Panel d: There was a shorter latency to enter chambers containing stimulus hamsters or sunflower seeds compared to empty chambers at 113g, 227g, 340g and reinstatement of 113g door weights.

Door weights had an effect on social or sunflower seed preference score ($p<0.001$, $F(7,98) = 13.267$) as revealed by a 2 x 8 mixed measures ANOVA. As weights increased, preference scores decreased ($p<0.05$: data not shown). There were no significant between group differences in social or sunflower seed preference score ($p=0.174$, $F(1,14) = 2.052$).

Door weights had an effect on time spent pushing the doors open ($p<0.001$, $F(5,55) = 10.895$), as revealed by a 2 x 2 x 6 mixed measures ANOVA (Figure 2.5). There was also a main effect of door condition (doors leading to stimulus hamsters or sunflower seeds versus doors leading to empty chambers) on time spent pushing doors open ($p<0.001$, $F(1,11) = 80.109$). Yet there was no significant between group differences in the time spent pushing the doors open ($p=0.490$, $F(1,11) = 0.510$). There was an interaction of door condition and door weights on time spent pushing the doors open ($p=0.033$, $F(5,55) = 2.640$). Subjects spent more time pushing the doors open at 340g, 454g, 634g and 794g compared to 113g weights as revealed by pairwise comparisons ($p<0.05$).

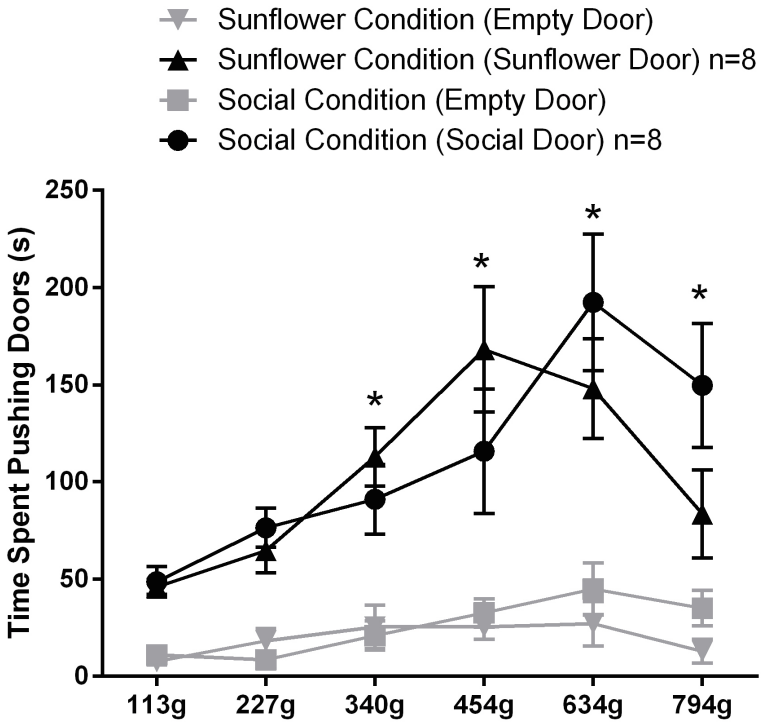


Figure 2.5: Effect of door weights on the time spent pushing the doors open.

As door weights increased the time spent pushing the doors for entry into stimulus chambers increased, but not doors for entry into empty chambers (* indicates difference in within-subjects door weights on time spent pushing the door open).

2.5 Discussion

These data indicate that this novel operant social preference task is a valid measure of social reward and social motivation. Opportunity for social interaction reinforces movement through a vertical-swing door. Hamsters rapidly acquired a preference for the chamber that provided social interaction over an empty chamber, in most cases requiring only 1-2 test sessions. Subjects also dramatically reduced their latency for performing the operant task of entry through a weighted chamber door by the second test session. Furthermore, a conventional

rewarding stimulus of presumably palatable food (sunflower seeds) also reinforced chamber entries in a manner similar to social interaction. Finally, progressively increasing the weight of the chamber door decreased the number of chamber entries, as expected, while increasing the time spent pushing the door open. In summary, this task provides a new approach for assessing reward and motivation.

The present operant task is independent of memory, as the doors allow visual, auditory and olfactory communication, and the task allows for the assessment of motivation under varying energy expenditure conditions. This method also allows for estimation of energy expenditure (cost) for rewarding stimuli by measuring the work required to push the door (potential energy of the door). Although still controversial and often debated, motivation can be operationally defined as the set of energetic forces that initiates work-related behaviors, determining the form, direction, intensity, onset and duration of emitted behaviors (Lorenz, 1950; Hogan, 1997; Graham and Weiner, 2006; Walton et al., 2006; Elliot, 2008; Pinder, 2008; Caldwell and Albers, 2015; Kim et al., 2017). Thus, with this OSP task, social motivation can be calculated using a set of dependent measures including latency to enter chamber, number of chamber entries, chamber preference score, the time spent pushing doors open and energy expenditure. These measures provide the opportunity to estimate reward value by measuring the relationship between rewards acquired and cost requirements (door weights). Additional highlights of this novel behavioral task are notable. 1) Social preference and social behavior are stable across consecutive days of testing (maintenance). 2) Removal of stimulus hamster results in a steady decrease in entries into chambers containing stimulus hamsters but not empty chambers (extinction), and reintroduction of stimulus hamster reinstates entries into social chamber and social preference (reinstatement). 3) Similar to Bailey et al. 2015 progressive hold-down task,

the OSP task can dissociate between the goal-directed components of motivation vs. general arousal (Bailey et al., 2015). At heavy door weights, rewards are accessed only through prolonged and continuous periods of goal-directed effort. Increases in goal-directed motivation are easily measured as increased time spent pushing doors to loaded stimulus chambers, but not doors to empty chambers. In contrast, increases in general arousal may increase time spent pushing any doors. 4) Finally, sunflower seeds reinforced entries into chambers in the absence of prior food deprivation, suggesting that the seeds have high hedonic value, although nutritional value may also contribute. Observation of hamsters during the testing revealed that they often retained the seeds in their mouth pouches as well as consuming the sunflower seeds immediately.

As with any experimental procedure, this one does have limitations. First, the interval between completion of operandum and presentation of the resultant stimulus is not and cannot be varied. Second, the number of entries through chamber doors (rewards acquired) particularly under high weights may depend on the subject's body weight and strength (although time spent attempting to push the door open serves as a complementary measure for motivation that should be less dependent on subject strength). Third, this apparatus is not automated. An experimenter must be present at all times to return subjects to the main chamber after entry into small chambers. Finally, in the present conditions, hamsters were socially deprived prior to testing, but were not food restricted. Future experiments can address at least this last caveat.

Due to the critical role of social motivation in the development and maintenance of beneficial social relationships, understanding the neural mechanisms controlling social reward and social motivation remains a high priority. The novel operant task reported here is a more direct measure of social reward and social motivation compared to the more traditional CPP paradigm because it is independent of social memory, directly quantifies consumption of reward,

and subjects have the choice to access or not access the social stimulus. This is the first operant task to use progressively weighted doors to assess social motivation, and the first to systematically quantify an operational definition of energy expenditure for reward (e.g. time spent pushing the door open). In summary, this operant task allows for a detailed assessment of social reward and social motivation, and presents great potential for use in identifying the neural mechanisms for these phenomena, as well as for studies of the rewarding properties of non-social stimuli.

3 CHAPTER THREE: ROLE OF OXYTOCIN IN THE VENTRAL TEGMENTAL AREA ON THE REINFORCING PROPERTIES OF SOCIAL INTERACTIONS

Note: this work has been published with contributions from co-authors: Kymberly N. Grantham, Lauren M. Aiani, Kyle J. Frantz, H. Elliott Albers and anonymous reviewers

(Borland et al. 2018)

3.1 Abstract

The rewarding properties of social interactions play a critical role in the development and maintenance of social relationships, and deficits in social reward are associated with various psychiatric disorders. In the present study, we used a novel Operant Social Preference (OSP) task to investigate the reinforcing properties of social interactions under conditions of high or low reward value, and high or low behavioral effort in male Syrian hamsters. Further, we investigated the role of oxytocin (OT) in a key structure of the mesolimbic reward system, the ventral tegmental area (VTA), in mediating the reinforcing properties of social interaction. Adult male hamsters were placed in a three-chambered apparatus, and allowed access to either a social chamber containing an unrestrained conspecific or a non-social chamber, by pushing through a one-way entry, vertical-swing door. Increasing the duration of social interaction (reward value) decreased the frequency of entering the social interaction chambers, whereas decreasing the duration of social interaction conversely increased the frequency of entries. Moreover, increasing behavioral effort required to access social interaction decreased the frequency of entries, especially under conditions when the duration of social interaction was only 5 seconds. OT injected into the VTA decreased the frequency of entering social interaction chambers in a manner similar to that observed when duration was increased, whereas injection of an OT receptor antagonist in the VTA increased the frequency of seeking social interaction.

Taken together, these data support the hypothesis that activation of OT receptors in the VTA are critical for the reinforcing properties of social interactions. Furthermore, social interactions may exhibit duration and cost dependent reinforcing effects on behavior similar to those observed with food and drugs of abuse.

3.2 Introduction

Social reward is a critical element in the development, expression, and maintenance of social behaviors and relationships (Suomi et al., 1971, Krach et al., 2010, Trezza et al., 2011). Despite the importance of social reward in adaptive and maladaptive behaviors, much remains to be learned about the reinforcing properties of social interaction and the neurobiological mechanisms underlying its rewarding properties. Various types of social interactions can serve as behavioral reinforcers (Everitt et al., 1987, Lee et al., 2000, Matthews et al., 2005, Trezza et al., 2011). To extend our ability to investigate social reward and reinforcement, we developed a new operant social preference (OSP) task for Syrian hamsters (Borland et al., 2017), a species in which social interactions are highly rewarding (Meisel and Joppa, 1994, Gil et al., 2013) and which serves as an important model for pre-clinical studies of psychiatric disease (Terranova et al., 2016). Hamsters are provided the choice to push through a vertical swing door into a chamber for brief social interaction, push into an empty chamber, or remain in a start chamber. As in other new operant social tasks (Cummings and Becker, 2012, Achterberg et al., 2016, Golden et al., 2017), animals demonstrated a preference for the social chamber within 2-3 test sessions, in a manner similar to preference for access to reward of a different modality, palatable food (sunflower seeds) (Borland et al., 2017). With this novel method, the parameters and neural underpinnings for behavioral reinforcement by social reward can be examined in great depth.

In the present study, we aimed to test the effects of both reward *value* and behavioral *cost* on the reinforcing effects of social interactions. Specifically, we investigated whether the duration of social interactions between adult males alters the motivating and reinforcing properties of those interactions. Because studies investigating the rewarding properties of other stimuli (e.g., drugs) have found that increasing reward value (e.g., dose) decreases the number of

rewards obtained in a test session (Maldonado et al., 1993, Doherty et al., 2013), we predicted that increasing the duration of social interaction would decrease the frequency of seeking social interaction. Conversely, decreasing the duration of social interaction would increase the frequency of seeking social interaction. Moreover, based on behavioral economics and progressive ratio schedules of reinforcement in drug and food reinforcement studies (e.g. (Rowlett, 2011, Beeler et al., 2012, Doherty and Frantz, 2012, Bentzley et al., 2014), we also predicted that increasing the behavioral cost (i.e., weight of an access door) to gain access to social interaction would decrease the frequency of seeking social interactions in a test session, especially when the duration of social interaction was relatively short. As such, the present study investigates whether the relationships between reward value (i.e. duration of interaction) and the effort required (i.e. weight on the vertical swing door) to obtain rewards are similar to those reported for other rewarding stimuli (e.g., drugs of abuse).

In a second set of experiments, we investigated the role of oxytocin (OT) in mediating the reinforcing properties of social interactions. OT is a neuropeptide that influences many different social processes and behaviors (Caldwell and Albers, 2016, Johnson and Young, 2017), including play behavior (Bredewold et al., 2014), social recognition (Albers, 2012, Wacker and Ludwig, 2012), and aggression (Harmon et al., 2002, Kelly and Goodson, 2014). Studies in humans also support a role for OT in regulating social behavior (Groppe et al., 2013, Rilling et al., 2014), and OT has been proposed as a potential treatment for a range of psychiatric disorders including autism spectrum disorder (Dichter et al., 2012, Stavropoulos and Carver, 2013, Young and Barrett, 2015). Because of the strong link between deficits in social reward and psychiatric disorders, understanding how OT contributes to the neural mechanisms controlling social reward is a critical gap in current knowledge.

OT administered peripherally or in the cerebroventricular system increases social reward in the context of conditioned place preference models (CPP) (Liberzon et al., 1997, Kent et al., 2013, Kosaki and Watanabe, 2016). These effects may be mediated by OT in the mesolimbic reward system. Activation of oxytocin receptors (OTRs) in the nucleus accumbens appears necessary for social reward in male mice (Dolen et al., 2013), and activation of OTRs in the ventral tegmental area (VTA) also appears necessary for social reward in male hamsters and mice (Song et al., 2016, Hung et al., 2017). Yet in these studies, the possibility that activation of OTRs influences the memory of the reward stimulus instead of the reward value itself cannot be excluded (Bardo and Bevins, 2000). Indeed, OT can influence memory processes in general and social memory in particular (de Wied and Versteeg, 1979, Albers, 2012, Gabor et al., 2012). Therefore, we used a direct test of social reinforcement, per se, with the operant social preference task, focusing on the role of OTRs in the VTA. We predicted that if activation of OTRs in the VTA mediates the reinforcing properties of social interactions, then injection of OT into the VTA will decrease the frequency of seeking social encounters, whereas injection of an OTR antagonist will increase the frequency of seeking social encounters.

3.3 Material and Methods

Subjects

Male Syrian hamsters (N=73, 11 wks of age; 120-140 g) were purchased from Charles River Laboratory (Wilmington, MA) and housed singly, in solid-bottom, Plexiglas cages (43 x 22 x 20 cm), containing corncob bedding and cotton nesting material (Neslets; Ancare, Bellmore, NY) in a humidity and temperature controlled (22^{o}C) vivarium. Hamsters were provided food and water *ad libitum,* and housed on a reverse light-dark (LD) cycle (14L:10D; lights off at

13:00) for 4 weeks before experiments. All behavioral tests were performed under red light during the first 3 hr of the dark phase. All procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and approved by the Georgia State University Institutional Animal Care and Use Committee.

Operant Social Preference (OSP) Apparatus

For a detailed description of the OSP apparatus, see (Borland et al., 2017). Briefly, the test apparatus consisted of three chambers: a main chamber, and two smaller chambers, each separated from the main chamber by one-way entry vertical-swing doors equipped with buckets that can hold weights (Figure 3.1). Test and stimulus hamsters can be placed in any of the chambers. The entire apparatus is made of clear Plexiglas with an open top, allowing detection of visual, olfactory and auditory cues among the chambers, thereby reducing memory requirements in the choice of whether or not to enter through the swing doors, e.g. to interact with a conspecific.

Figure 3.1: Operant Social Preference Apparatus: schematic (left) and vertical view (right).

The main chamber contains an experimental subject whereas, one of the side chambers is occupied by a stimulus hamster; the subject and stimulus hamster are separated by a vertical-swing door. Visual, auditory and olfactory cues are shared between the chambers through the open top and holes in the door. An acrylic bucket positioned on the stimulus hamster side of the doors serves to hold weights.

Operant Social Preference (OSP) Conditioning

For a detailed description of conditioning sessions, see (Borland et al., 2017). Briefly, male test hamsters (120-140 g) were allowed to move throughout the apparatus, while a smaller (100-120 g) male stimulus hamster was confined to one of the small chambers. Stimulus hamsters were group-housed five per cage, and subjects were paired with a different stimulus hamster every test session. If a subject chose to enter a small chamber, time allowed in that chamber was either 20 sec (baseline), 5 sec, or 60 sec. After the designated time course expired, the subject hamster was removed from the small chamber by the experimenter and placed back into the main chamber, facing the back wall, equidistant from the two small chambers. All test sessions were 10 min in total length. To assess chamber entries under higher effort requirements after conditioning sessions, door weights were progressively increased over consecutive days, from baseline at 113g (4oz), to 227g (8oz), 340g (12oz), 454g (16oz) and 624g (22oz). Only subjects that met acquisition criteria (at least 2 entries into chambers containing stimulus hamsters per session and more entries into social chambers vs. non-social chambers, for at least 2 consecutive test sessions) were included in the analysis.

Behavioral Scoring

All behavioral tests were video recorded (Panasonic-WVCP294) and analyzed using the Noldus Observer system (11.5, Leesburg, VA). A scorer blind to treatments scored each videotape for the following measures: the number of entries into chambers, latency to enter chambers (first latency and subsequent latencies, i.e. post reinforcement pause), social preference score (entries into chambers containing stimulus hamsters – entries into non-social chambers), and social entries per minute (number of social entries divided by the total time spent in the main chamber). Post reinforcement pause was calculated as the time to re-enter chambers containing stimulus hamsters or non-social chambers respectively once subjects are returned to the main chamber. The quality of social interaction was also scored: duration of aggression, social investigation, submission (e.g. fleeing, avoidance), grooming, and non-social behavior were considered mutually exclusive, and the proportions of time spent in each were calculated. The frequency of attacks was calculated based on point events during displays of aggression, and flank marks were scored as point events during non-social behavior. Flank marks were scored due to their strong link to dominance status and territoriality in rodents (Ferris et al., 1987, Terranova et al., 2017). For operational definitions of these behaviors, see (Drickamer et al., 1973, Gray et al., 2015). Locomotor activity was also scored as the number of entries into any of the six equal-sized squares (16.9 x 16.5 cm) into which the OSP apparatus was subdivided for analysis.

Stereotaxic Surgery

Hamsters were anesthetized with isofluorane (induced at 5% and maintained at 2-4%), a 4 mm 26-gauge cannula was implanted unilaterally and was aimed at the VTA (from bregma; anteroposterior (AP) -3.80 mm; mediolateral (ML) +0.55 mm; dorsoventral (DV) -3.20 mm; 0^{o}

angle). Previous studies investigating the effects of OT in the VTA on social reward have also used unilateral injections (Song et al., 2016, Hung et al., 2017). All hamsters were injected subcutaneously with the anti-inflammatory agent, ketoprofen (5mg/kg), and were allowed to recover for at least 4-6 days prior to behavioral testing.

Intra-VTA Drug Treatment

Microinjections were administered using a 12 mm, 32-gauge needle attached to a 1 µl Hamilton syringe that extended an additional 4.2 mm beyond the cannula to a final depth of 7.4 mm below the skull surface. Drug was delivered in a volume of 200 nl at a rate of 0.400 µl per min using an infusion pump (Harvard Apparatus, Holliston, MA). The needle was left in place for an additional 30 sec to allow diffusion away from the tip of the injection needle. Approximately 5 min after microinjection, hamsters were tested in the OSP apparatus. The drugs used were oxytocin (Bachem, CA, USA) dissolved in sterile saline to a final concentration of 9 µM or 90 µM; and desGly-NH_2-d$(CH_2)_5$[D-Tyr2,Thr4]OVT, a highly selective OTR antagonist (Manning *et al,* 2012; gift from Dr. Maurice Manning), dissolved in sterile saline to a final concentration of 0.9 µM or 9 µM. Drug doses were based on previous studies indicating effects on social CPP in Syrian hamsters (Song et al., 2014, Song et al., 2016).

Histology

Within 24 hr of the final behavioral tests, hamsters were euthanized with a lethal dose of sodium pentobarbital (0.25 ml, i.p. Henry Schein Animal Health, Dublin, OH), and 200 nl of India Ink was microinjected through the guide cannula to mark the injection site. Hits were

categorized if ink was seen within the caudal VTA, referenced to the hamster stereotaxic atlas (Morin and Wood, 2001).

Experimental Design and Statistical Analysis

Data were analyzed using SPSS software (SAS Institute, 1990, 23.0 for Windows). All data were examined to determine if the assumptions of parametric statistical tests were met (normality, equal variance, sphericity). When assumptions were violated, data were cube-root transformed (Exp. 1 the latency to re-enter chambers, Exp. 2 the number of entries into chambers, social preference score, social entries per minute in main chamber score Exp. 3 the duration of grooming and the number of flank marks). All tests were two-tailed, and results considered statistically significant if $p \leq 0.05$. All data are presented as mean ± standard error of the mean.

Experiment 1: Effect of the Duration of Social Interactions on Social Preference. After stable acquisition of a social preference (more entries into the chamber containing a social stimulus vs. the non-social chamber) at 113g door weights and 20 sec per entry, subjects (n=11) experienced different duration conditions: 5, 20 (baseline) or 60 sec in chambers per entry. Door weights were maintained at 113g. Assignment of duration was counter-balanced within-subjects, such that each subject experienced each duration for one session. A repeated measures one-way ANOVA was carried out to examine effect of the duration of social interaction on behavior. Two subjects failed to meet acquisition criteria.

Experiment 2: Interaction between Duration of Chamber Exposure and Door Weights on Social Preference. After stable acquisition of a social preference at baseline duration (20 sec) and door weights (113 g), subjects were assigned to experience 5 (n=9) or 60 (n=8) sec in chambers after every entry into social interaction and non-social chambers. The weights on doors were progressively increased over 5 consecutive days to 113 g (4 oz), 227 g (8 oz), 340 g (12 oz), 454 g (16 oz) and 624 g (22 oz). A 2 x 5 mixed measures, between-within ANOVA was carried out to test the effects of the duration of social interaction and the weights of the doors on behavior. Binomial distributions were also carried out to test for the effects of the duration of social interaction on the ability to successfully achieve entries into chambers versus not during a test session. Binomial distributions were compared by calculating z scores (test statistic). All subjects met acquisition criteria.

Experiment 3: Effect of OT or OTR Antagonist in the VTA on Social Preference. After stable acquisition of a social chamber preference, subjects were surgically implanted with a guide cannula aimed at the caudal VTA. Subjects received injections of saline, 9μM OT (n=18), and 90μM OT (n=14) in a counter-balanced order: other subjects received saline, 0.9μM OTR antagonist, and 9μM OTR antagonist (n=14) in a counter-balanced order. Injections and behavior testing occurred over 3 consecutive days. Since the high dose of OT (90μM) resulted in secondary behavioral effects (flank marking and grooming), subjects received injections of only saline or the low dose of OT (9μM) in subsequent experiments. In this experiment, subjects experienced 20 sec in small chambers per entry and door weights were 113g. Paired sample t-tests were carried out to examine drug effects on behavior (saline versus specific drug

treatments). Nine subjects had cannulas outside the caudal VTA and were not included in the analysis. Four subjects did not meet acquisition criteria.

3.4 Results

Experiment 1: Effect of Duration of Social Interactions on Social Preference.

The duration of social interactions influenced the number of entries into the social chambers (i.e., chambers containing a stimulus hamster), according to a main effect of duration ($p<0.001$, $F(2,20) = 25.278$) (Figure 3.2a). Specifically, 20 sec of social interactions following entry decreased the number of entries into chambers containing stimulus hamsters, compared to 5 sec of social interaction ($p=0.050$; 5 seconds 6.227 +/-0.740; 20 sec 5.091 +/-0.653). Furthermore, 60 sec of social interaction decreased the social chamber entries, compared to 20 sec ($p=0.001$; 60 sec 1.909 +/-0.241). On the other hand, the duration in stimulus chambers had no effect on the number of entries into the non-social chambers ($p=0.525$, $F(2,20) = 0.665$; Figure 3.2a). As expected, there were no differences in the latency to the first entry into chambers in groups assigned to different durations of time in the chambers containing a social stimulus ($p=0.482$; $F(2,20) = 0.600$) or to groups assigned to different durations of time in non-social chambers ($p=0.398$; $F(2,20) = 0.966$) (Figure 3.2b). There was, however, a significantly shorter latency to the first entry into chambers containing a stimulus hamster compared to the latency to the first entry into empty chambers ($p=0.002$; $F(1,20) = 16.667$) (Figure 3.2b inset).

The duration of social interactions influenced the social preference score, according to a main effect ($p=0.001$, $F(2,20) = 11.347$) (Figure 3.2c). Pairwise comparisons revealed that 60 sec interactions decreased social preference scores compared to 20 sec interactions ($p=0.010$: 60 seconds 1.045 +/-0.396; 20 seconds 4.000 +/-0.874) or 5 sec interactions ($p<0.001$; 5 seconds

4.773 +/-0.651), but no difference in social preference scores occurred between 5 sec and 20 sec social interactions (p=0.385). An important caveat to the design of this experiment is that although the test session was 10 minutes in length for all subjects, subjects that spent 60 seconds in chambers per entry spent less time in the main chamber, and thus had fewer opportunities to enter small chambers compared to subjects that spent 5 seconds in chambers per entry. To control for differences in time spent in the main chamber, social entries per minute in the main chamber scores were calculated. The number of entries into chambers with stimulus hamsters a subject made during the test session was divided by the time over which each subject had to choose to enter one or the other small chamber, i.e. time spent in the main chamber. Results were the same as the raw frequency scores, such that the duration of social interaction had a main effect on social entries per minute score ($p<0.001$, $F(2,20) = 13.444$; Figure 3.2d). Pairwise comparisons revealed that when subjects spent 60 seconds in chambers there was a slower rate of entering chambers containing stimulus hamsters (i.e., number of entries per minute) compared to when subjects had social interactions for 20 seconds (p=0.005; 60 seconds 0.278 +/-0.044) or 5 seconds (p=0.001). However, there was no difference in rate of entry into chambers containing stimulus hamsters between conditions in which subjects experienced 5 seconds and 20 seconds in chambers (p=0.895; 5 seconds 0.672 +/-0.085; 20 seconds 0.663 +/-0.102).

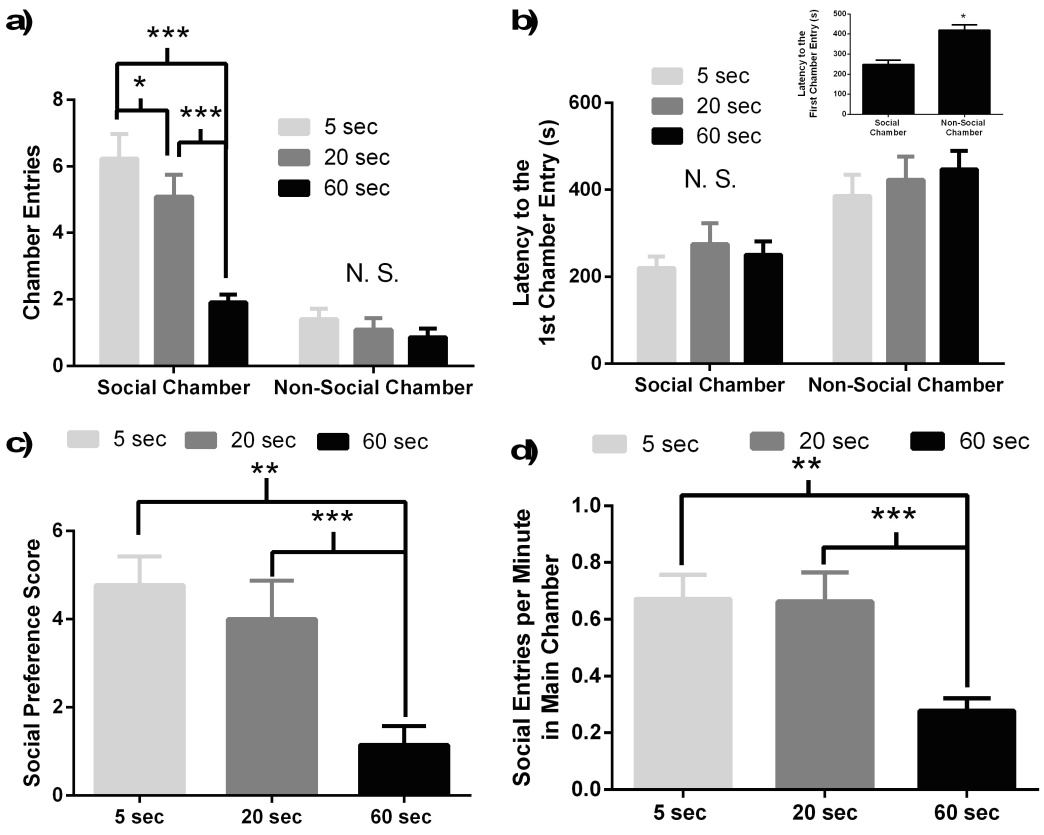


Figure 3.2: Effect of Time Allowed in Chambers Containing a Stimulus Hamster and Time Allowed in Non-Social Chambers on Social Preference.

a) More time allowed in chambers decreased number of entries into social chambers, but had no effect on entries into non-social chambers (n=11; * $p \leq 0.050$; *** $p \leq 0.001$). b) Time spent in chambers had no effect on the first latency to enter chambers. However, across duration conditions, subjects were quicker to enter chambers containing stimulus hamsters compared to non-social chambers (inset). c) More time in chambers decreased social preference score (** $p \leq 0.010$). d) More time in chambers decreased the rate of social chamber entries as a proportion of time in the main chamber.

Next, we examined whether the duration of time spent in the chambers had an effect on the latency to re-enter chambers following the first entry (post reinforcement pause). Spending 60 sec in the chambers containing stimulus hamsters increased ($p=0.003$, $F(2,20) = 8.080$) the latency to re-enter the social chambers compared to spending 20 sec ($p=0.033$) and 5 sec ($p=0.007$) in the chambers (Figure 3.3a). Spending 60 sec in the chambers containing stimulus hamsters also increased ($p=0.012$, $F(2,12) = 6.484$) the second post reinforcement pause (second latency to re-enter) compared to spending 20 sec ($p=0.010$) in the chambers. The duration of time in the chambers had no effect on the latency to re-enter the non-social chambers ($p>0.05$). Overall, increasing the duration of time spent in social stimulus chambers to 60 sec increased the latency to enter those chambers compared to 5 or 20 sec durations in the social chambers.

Finally, we explored the quality of social interactions in the social chambers, specifically by considering whether the duration of the time spent in the social chambers per entry influenced the nature of the interaction. The duration in the social chambers did affect the proportion of time spent in social investigation ($p=0.014$, $F(2,20) = 5.327$); such that the proportion of time spent in social investigation was lower in the 60 sec condition group compared to the 20 sec condition group ($p<0.001$) (Figure 3.3b). On the other hand, time in the social chambers had no effect on the proportion of time displaying aggression ($p=0.294$, $F(2,20) = 1.301$) nor the frequency of attacks ($p=0.166$, $F(2,20) = 1.966$). The 60 sec condition group did spend more time grooming ($p=0.032$) and displayed a higher rate of flank marking ($p=0.017$) (Figure 3.3c) compared to the 20 sec condition group when in the main chamber.

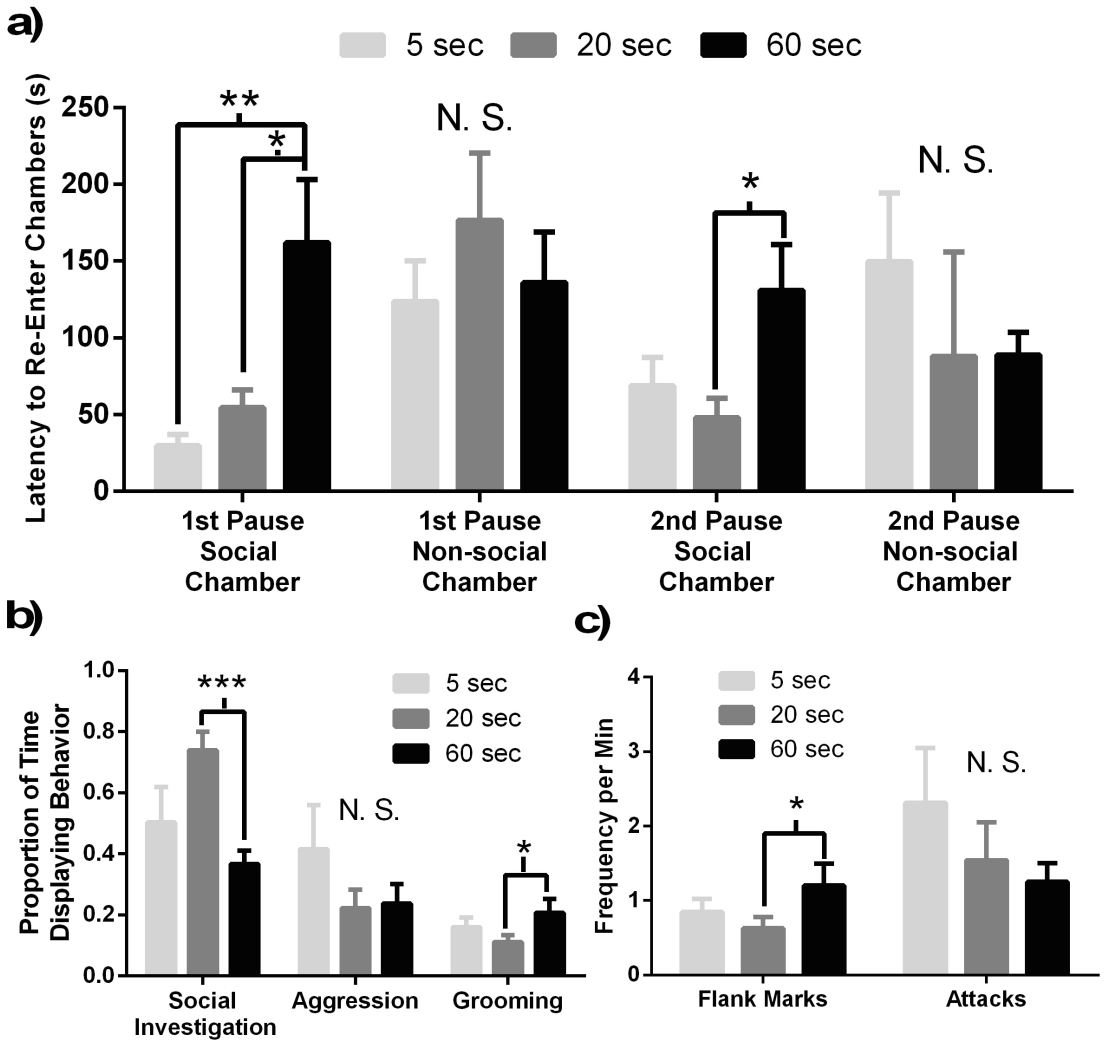


Figure 3.3: Effect of Time Allowed in Social and Non-Social Chambers on Social Behavior and Post Reinforcement Pause.

a) More time in the chambers increased the latency to re-enter the chambers containing stimulus hamsters, but had no effect on the latency to re-enter the non-social chambers (post reinforcement pause) (** $p \leq 0.010$). b) More time allowed in the chambers decreased the proportion of time spent socially investigating stimulus hamsters when in the social interaction chambers, but an increase in the proportion of time spent grooming when in the main chamber (n=11; * $p \leq 0.050$; *** $p \leq 0.001$). c) More time allowed in the social interaction chambers also increased the rate of flank marking when in the main chamber.

Experiment 2: Interaction between Duration of Social Interaction and Behavioral Cost on Social Preference

Because a decrease in the frequency of the number of rewards obtained could be interpreted as either an increase (less rewards needed for satiety) or a decrease in reward value (less motivation for reward); in the following study we investigated the effects of varying the behavioral cost of obtaining the reward on social preference between the two reward conditions (i.e., 5 or 60 sec of social interaction) to help discriminate between these alternative possibilities. While all hamsters entered the chambers containing stimulus hamsters when the door weighed 113g or 227g, significantly fewer hamsters ever entered the social chambers as the door weights increased (z score = 4.246, $p<0.001$) (Figure 3.4a). Interestingly, however, consistent with the possibility that a longer duration of time in the social chambers was more rewarding, significantly more hamsters entered the social chamber at least once in the group given 60 seconds in the social chamber compared to the group given 5 seconds in the chamber when the doors weighed 340g, 454g, and 624g (z score = 1.917, $p=0.027$) (Figure 3.4a). An interaction between the duration of social interaction and the weight of the entry door (i.e., behavioral cost) on the total number of entries into social chambers was also observed ($p=0.005$, $F(4,60) = 4.132$). Progressively increasing the weight of the door decreased social entries when the duration in the chambers was 5 sec, such that 227g ($p=0.002$), 340g ($p=0.002$), 454g ($p<0.001$), and 624g ($p<0.001$) all resulted in fewer entries than at baseline (113g). However, when the duration in stimulus chambers was 60 sec, only the three heaviest weights (i.e., 340g ($p=0.030$), 454g ($p=0.019$) and 624g ($p<0.001$)) significantly decreased the number of social chamber entries below baseline (113g) (Figure 3.4b). The duration of social interaction interacted with

weight of the door to influence social preference score as well ($p=0.006$, $F(4,60) = 3.996$) (Figure 3.4c). For subjects that experienced 5 sec in chambers, all door weights decreased social preference score (227g ($p=0.028$), 340g ($p=0.003$), 454g ($p<0.001$), and 624g ($p<0.001$)). However, for subjects that received 60 sec in chambers, only the heaviest door weight (624g) was effective ($p=0.021$) in decreasing the social preference score (Figure 3.4c). With regard to social chamber entries per minute in the main chamber, duration of social interaction and door weight also interacted to influence outcomes ($p=0.049$, $F(4,60) = 2.540$). For subjects that were allowed 5 sec social interactions, all heavier weights decreased the social chamber entries per minute: 227g ($p=0.003$), 340g ($p=0.001$), 454g ($p<0.001$), 624g ($p<0.001$). However, for subjects that received 60 sec social interactions, only 340g ($p=0.030$), 454g ($p=0.018$) and 624g ($p=0.001$) door weights decreased the social chamber entries per minute (Figure 3.4d).

To examine the relationship between the number of entries into social chambers and door weights, simple linear regressions were calculated. Social entries correlated negatively with door weights, both for subjects allowed 5 sec social interactions ($F(1,3) = 22.516$, $p=0.018$; R^2 of 0.882) and those allowed 60 sec social interactions ($F(1,3) = 29.727$, $p=0.012$; R^2 of 0.908). Comparison of slopes between the 5 and 60 sec condition correlations revealed a z score of 3.638 ($p<0.001$). Thus, the rate at which increasing the weight of the chamber doors (behavioral cost) decreased the number of entries (rewards acquired) was greater in 5 sec social interaction conditions, compared with 60 sec interaction conditions (Figure 3.4e). There was no correlation between door weights and entries into the non-social chambers for 5 sec group ($p=0.229$, $R^2=0.430$) nor the 60 sec group ($p=0.413$, $R^2=0.069$).

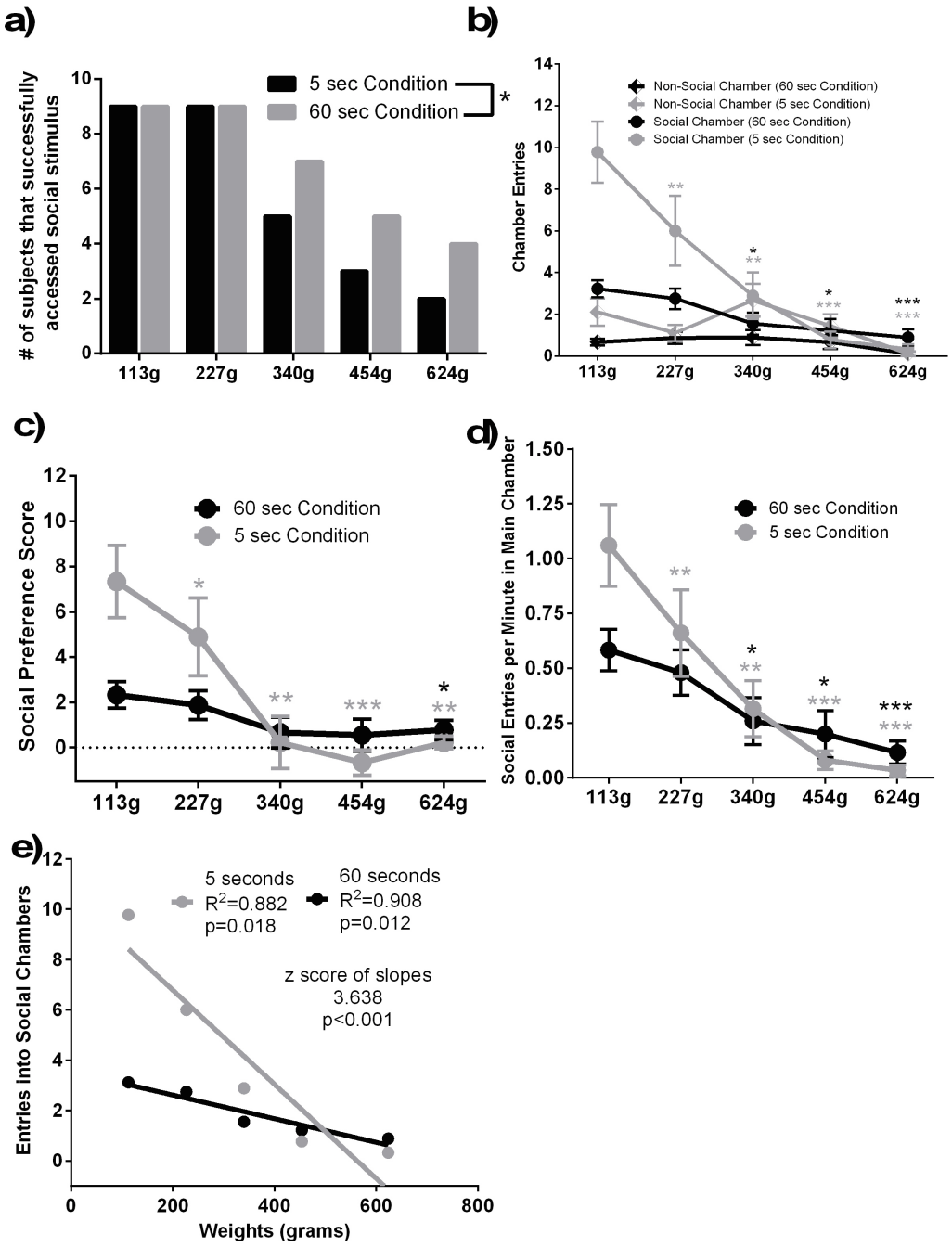


Figure 3.4: Effect of the Duration of Social Interaction on Social Preference under Conditions Requiring Increasing Effort.

Asterisks in graphs (b, c, d) indicate a significant difference from the door weight of 113g, with asterisks and lines color-coded (gray 5 sec Condition; black 60 sec Condition) (* $p \leq 0.050$; **

p≤0.010; *** p≤0.001). a) While all hamsters (n = 9) entered the chambers containing stimulus hamsters when the door weighed 113g or 227g, significantly fewer hamsters entered the social chambers at least once as the door weights increased (n = 9; * p < 0.05). Significantly more hamsters in the 60 sec group entered the social chamber at least once in the session compared to the 5 sec duration group (p = 0.05). b) All door weights (compared to 113g) decreased the number of entries into chambers containing stimulus hamsters for subjects that were allowed 5 seconds of social interaction (n=9). Only the heaviest three door weights decreased the number of entries into chambers containing stimulus hamsters for subjects that were allowed 60 sec of social interaction (n=8). c) Compared to a door weight of 113g, all door weights decreased the social preference score for subjects that were allowed 5 sec of social interaction. Only the heaviest door weight decreased social preference score for subjects that were allowed 60 seconds of social interaction. d) Compared to a door weight of 113g, all door weights decreased the number of entries per minute for subjects allowed 5 sec of social interaction. Only the heaviest 3 door weights decreased social entries per minute in the main chamber for subjects that were allowed 60 sec of social interaction. e) Correlation between door weights and the number of entries into chambers containing stimulus hamsters revealed that both 5 sec (n=9) and 60 sec (n=8) treatment conditions show a linear decrease in number of entries with increasing door weights. The slope is greater in the 5 sec condition than the 60 sec condition.

Experiment 3: Effect of OT and OTR Antagonist Injected into the VTA on Social Preference.

OT (9μM or 90μM) injected into the caudal VTA decreased the number of entries into chambers containing stimulus hamsters, compared to saline (p<0.001, t(17) = -4.389; 9μM OT 3.11 +/-0.411, saline 4.67 +/-0.524; p=0.041, t(13) = -2.271; 90μM OT 2.21 +/-0.793, saline 3.86

+/-0.443; Figure 3.5a). Conversely, injections of 0.9μM OTR antagonist into the VTA increased the number of entries into chambers containing stimulus hamsters (p=0.006, t(13) = 3.214; OTR antagonist 5.33 +/-0.52, saline 3.87 +/-0.60; Figure 3.5b). Neither OT nor the OTR antagonist affected the number of entries into non-social chambers (p>0.050). The latency to re-enter the chamber containing the stimulus hamsters was longer for hamsters given 90 μM OT compared to saline controls although this difference did not reach statistical significance (p=0.132, t(7) = 1.706; Figure 3.6a). Similarly, the latency to re-enter the social chambers following administration of the OTA at 9 μM was shorter than in saline controls although this difference also did not reach statistical significance (p=0.096, t(11) = -1.822; Figure 3.6b).

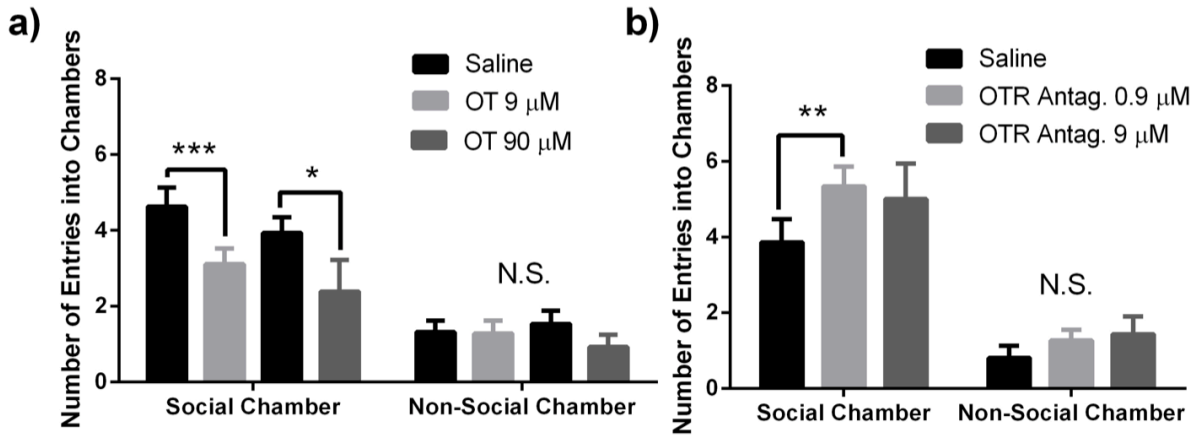


Figure 3.5: Effect of injection of OT and an OTR Antagonist in the VTA on Social Preference. a) OT (9 μM n=18; 90 μM n=14) injected in the VTA 5 minutes before the test decreased the number of entries into chambers containing a stimulus hamsters (i.e., Social Chamber), but had no effect on the number of entries into non-social chambers (i.e., non-social chamber; * p≤0.050; *** p≤0.001). b) Injection of the OTR antagonist at 0.9 μM (n=14) but not 9 μM (n=14) increased the number of entries into the Social Chamber, but neither dose affected the number of entries into the non-social chamber (** p≤0.010).

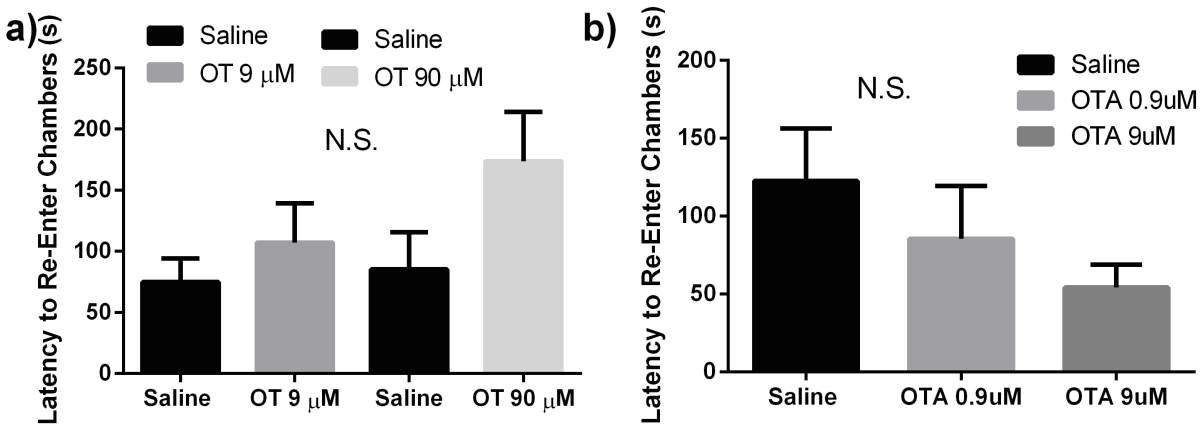


Figure 3.6: Effect of Injections of OT and an OTR Antagonist in the VTA on the latency to re-enter chambers containing stimulus hamsters (post reinforcement pause).

a) OT (90 μM) injected into the VTA increased the latency to re-enter chambers containing stimulus hamsters trended towards significance (p=0.132). b) Likewise, injections of the OTR antagonist (9 μM) decreased the latency to re-enter chambers containing stimulus hamsters also just missed significance (p=0.096).

Neither OT nor the OTR antagonist affected social investigation or aggression (proportion of time displaying social investigation and aggression (Figure 3.7a, b) nor the rate of attacks per minute (Figure 3.7c, e). There was a trend for the 90μM concentration of OT to increase the duration of grooming (p=0.066, t(11) = 2.042; OT 80.8 +/-34.5, saline 32.5 +/-11.8), but there was no effect of the OTR antagonist on these behavioral measures compared to saline (Figure 3.7d, f). The 90μM concentration of OT increased the number of flank marks observed (p=0.043, t(11) = 2.288; OT 12.17 +/-4.78, saline 2.25 +/-0.94), although the OTR antagonist had no effect compared to saline (Figure 3.7g, i). The 0.9μM concentration of the OTR antagonist significantly increased the amount of locomotor activity (p=0.021, t(13) = 2.595; OTR antagonist 120 +/-7, saline 102 +/-7) compared to saline. There was also a trend for the 9μM

concentration of the OTR antagonist to increase the amount of locomotor activity (p=0.061, t(13) = 2.047; OTR antagonist 118 +/-11) compared to saline (Figure 3.7h).

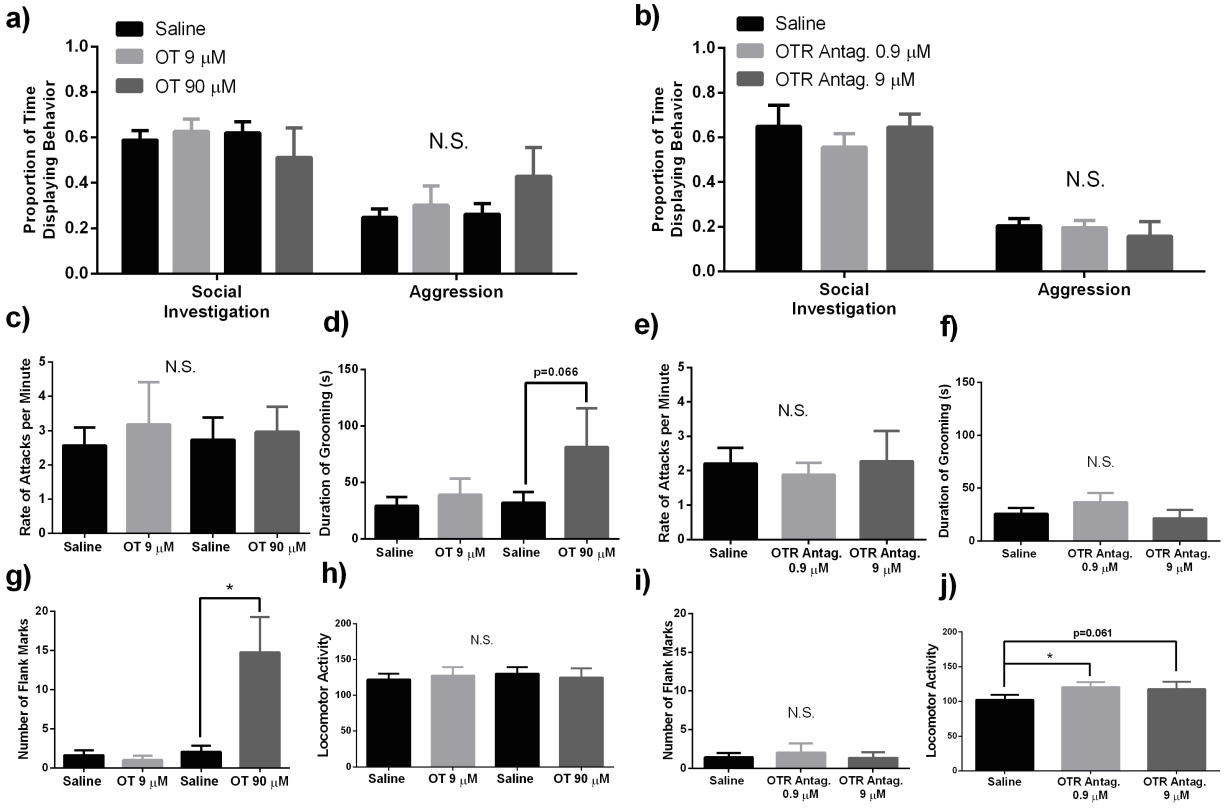


Figure 3.7: Effect of injection of OT and an OTR Antagonist in the VTA on Social Investigation, Aggression, Grooming and Flank Marking, and Locomotor Activity.

a) OT (9 μM n=18; 90 μM n=12) injected into the VTA had no effect on social investigation, aggression or c) attacks per minute. b) Injection of the OTR antagonist (0.9 μM n=14) had no effect on social investigation, aggression or e) attacks. d) A trend for the 90 μM concentration of OT to increase the duration of grooming that just missed significance (p=0.066) was observed. f) Injections of the OTR antagonist had no effect on grooming. g) The 90 μM concentration of OT increased the number of flank marks (* p≤0.050). i) The OTR antagonist injected into the VTA had no effect on the number of flank marks observed. h) Injection of OT had no effect on the amount of locomotor activity. j) Injection of 0.9 μM OTR antagonist increased locomotor

activity ($p \leq 0.050$) and a trend for 9 μM OTR antagonist to increase locomotor activity just missed significance ($p=0.061$).

Histological analysis of injection sites, which can be seen in Figure 3.8, revealed 9 subjects for which injections were outside the caudal VTA. No drug effects were observed among the nine hamsters for which injection sites were found to be outside of borders of the caudal VTA (data not shown).

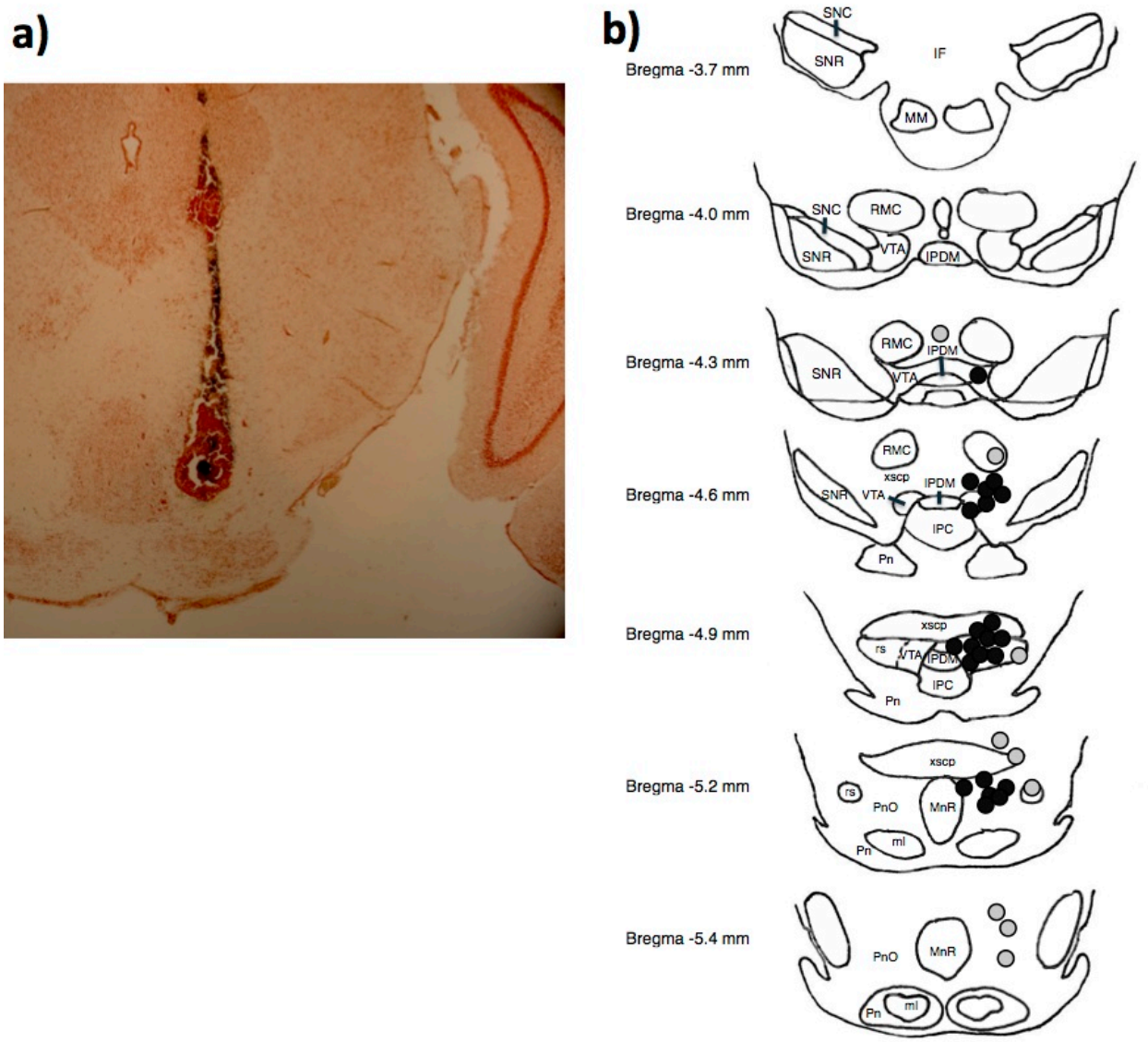


Figure 3.8: Histology of Drug Injections.

a) Representative picture of injection site to the caudal VTA. b) Localization of the sites of injection of oxytocin (OT) and OT receptor antagonist. Subjects with ink found within the caudal VTA were classified as hits (black circle), while subjects with ink found outside the caudal VTA were classified as misses (gray circle). IF: interfascicular nucleus IPC: interpeduncular nucleus caudal IPDM: interpeduncular nucleus dorsomedial ml: medial lemniscus MM: medial mammillary nucleus MnR: median raphe nucleus Pn: pontine nucleus PnO: pontine reticular nucleus oral RMC: red nucleus rs: rubrospinal tract SNC: substantia nigra compact SNR: substantia nigra reticular VTA: ventral tegmental area xscp: decussation of the superior cerebellar penduncle.

3.5 Discussion

From the present findings that increasing the duration of time allowed in the stimulus chambers decreases the number of entries and increases the latency to re-enter those chambers (post reinforcement pause), we infer a more general conclusion that this operant social preference task reveals the expected relationship that increasing reward value decreases the number of rewards obtained. We interpret the findings that increasing the weights on the doors decreases the number of entries into chambers containing a stimulus hamster, as an indicator that increasing behavioral effort required to obtain rewards will decrease the number of rewards obtained. Moreover, reward value and behavioral effort interacted in predictable ways, such that the influence of behavioral cost was greater in conditions of lower reward value than higher reward value. Finally, the present results also supported a role for OT within the VTA in these relationships. OT injected into the caudal VTA decreased the number of entries into chambers containing stimulus hamsters, and did so in the same manner as increasing the duration of time in

the stimulus chambers. Conversely, an OTR antagonist injected into the VTA increased the number of entries into chambers containing stimulus hamsters, in the same manner as decreasing the time in the stimulus chambers. There was also a trend that OT increased the latency to re-enter social chambers and that the OTR antagonist reduced the latency to re-enter chambers.

In general, none of the manipulations of reward value, behavioral effort, or neural activity affected entries into non-social chambers. The effects of OT and the OTR antagonist on other behaviors were not statistically significant except that the high concentration of OT induced flank marking and increased the duration of grooming; an effect that may be due to the activation of arginine-vasopressin V1a receptors (Song and Albers, 2017). This is particularly interesting considering that there was also an increase in the proportion of time spent flank marking and grooming in the main chamber for subjects that experienced 60 sec of interaction per entry. There was also a decrease in the proportion of time spent socially investigating the stimulus hamster for subjects that experienced 60 sec of interaction. However, these effects may be due to differences in the total time in the main chamber versus social interaction chamber between the different duration conditions. In other words, although there was a decrease in the proportion of time spent per entry socially investigating for subjects that experienced 60 sec of interaction, the total time spent socially investigating was greater compared to subjects that experienced 20 sec and 5 sec. Subjects that experienced 60 sec in the chambers per entry spent on average 120 sec in the social interaction chambers per test session, while subjects that experienced 20 sec spent on average 60 sec in the social chambers, and the 5 sec group spent on average only 30 sec in the social chambers. Likewise, the increase in the proportion of time spent grooming and flank marking for subjects that experienced 60 sec may be due to a decrease in the total time in the main chamber.

We also found that OTR inactivation in the VTA increases locomotor activity. Previous studies have reported similar effects of OTR activation and inactivation in the substantia nigra in male and female rats (Angioni et al., 2016, Leong et al., 2016). However, the OTR antagonist did not increase the number of entries into the non-social chamber. To our knowledge, this is the first study to show that OT injected into the VTA is sufficient to modulate the value of social reward in a behavioral reinforcement paradigm, or that OTR activation in the VTA is critical for normal expression of such behaviors. Furthermore, OT's effects on social reward are likely due to activation of OTRs and not V1aRs in the VTA (Song et al., 2016). Taken together, these data support the hypothesis that OT in the VTA regulates social reward and reinforcement.

The operant task used in the present study provides a rich set of dependent measures of reward and motivation including the number of entries into chambers, the first latency to enter chambers, subsequent latencies to re-enter chambers and chamber preference score (Borland et al., 2017). Also unique to this operant task, adding weights to the doors provides a powerful approach for increasing the behavioral cost to access a social stimulus. Finally, the simplicity of the apparatus and utility of the two small chambers can support a myriad of economic decision models for the study of social motivation or other rewarding modalities.

A main goal of this study was to determine whether the same experimental parameters regulate social reinforcement in this operant social preference task, as influence drug and food reinforcement in classic drug and food self-administration studies. Both our prior report and the present results suggest that they do. In (Borland et al., 2017), we noted palatable food (sunflower seeds) reinforces entries into chambers to a similar intensity as social interactions, and removal of the social stimulus decreases entries into chambers. Here, we demonstrate further that when the social stimulus is present, there is an inverse relationship between duration

of social interaction and frequency of entries into social chambers. Indeed, substantial evidence demonstrates an inverse relationship between reward value and reward consumption (e.g. cocaine dose and cocaine infusions) (Maldonado et al., 1993, Veeneman et al., 2012, Doherty et al., 2013). We also report a direct relationship between duration of social interaction and post reinforcement pause (latency to re-enter social interaction chamber). Additional studies over many decades have demonstrated the inverse relationship between behavioral cost and reward consumption as well (Salamone et al., 2009, Bentzley et al., 2013). Furthermore, we demonstrate that the correlation between behavioral cost and reward consumption interacts with reward value, such that reward consumption declines at faster rates with increased behavioral cost if the reward value is relatively low. These outcomes fit well with models of behavioral elasticity (Salamone et al., 2009, Rowlett, 2011), all of which stem from interpretations based in behavioral economics.

Finally, injections of OT or OTR antagonist into the VTA produce results similar to classic manipulations of dopamine signaling in the mesolimbic circuitry. For example, administration of a dopamine receptor antagonist (SCH 23390) into the nucleus accumbens, amygdala or striatum increased cocaine self-administration (Maldonado et al., 1993, Caine et al., 1995). At certain concentrations, dopamine perfusate in the nucleus accumbens decreases cocaine self-administration (Hurd and Ponten, 2000). Enhancement of OT signaling by direct OT administration to VTA appears similar to enhancing dopamine transmission, whereas blocking OT receptors produced results similar to blocking dopamine transmission. For example, OT injected into the caudal VTA decreased sucrose consumption, while an OTR antagonist injected into the caudal VTA increased sucrose consumption in rats (Mullis et al., 2013). Notably, experimental conditions that decrease the number of rewards obtained in an

operant task can be interpreted as an increase or a decrease in reward value, and various schedules of reinforcement are often used to differentiate between these possibilities. Although we provide evidence that the reduction in operant responding in response to OT administered in the VTA in the present study is consistent with an increase in social reward value, it remains possible that these findings could be interpreted as OT reducing value (Peters et al., 2017). Future studies using various schedules of reinforcement could be used to help discriminate between these interpretations. Together, our results suggest that activation of OTRs in the VTA modulates reward value to influence reinforced behavior in this social preference task.

Although beyond the scope of the present experiment, circuit analyses suggests that inputs from OT-containing neurons in the paraventricular nucleus into the VTA may be critical for social reward in adult male mice (Hung et al., 2017). Furthermore, projections from the VTA to the nucleus accumbens regulate social behavior (Gunaydin et al., 2014). In terms of mechanisms by which OT influences dopamine transmission in this pathway, OT receptors are located on dopamine and glutamate-containing neurons in mice (Peris et al., 2017) and injections of OT in the caudal VTA increased extracellular dopamine in the nucleus accumbens (Melis et al., 2007).

In conclusion, these data support the hypothesis that OT can enhance social reward (Groppe et al., 2013, Feng et al., 2015, Chen et al., 2017) via activity in the mesolimbic dopamine system (Melis et al., 2007), consistent with clinical research. Furthermore, economic demand models of motivation can serve as important tools for identifying promising addiction treatments (Bentzley et al., 2014), such as OT administration (Cox et al., 2017). As such, drugs that activate OTRs and thereby modulate social motivation may represent an approach that will contribute to the development of new treatments for psychiatric and mental health disorders

(Meyer-Lindenberg et al., 2011, McGregor and Bowen, 2012, Caldwell and Albers, 2016), such as Autism Spectrum Disorder (Stavropoulos and Carver, 2013, Gordon et al., 2016).

4 CHAPTER FOUR: SEX DEPENDENT REGULATION OF SOCIAL REWARD BY OXYTOCIN RECEPTORS IN THE VENTRAL TEGMENTAL AREA

Note: this work has been published with contributions from co-authors: Lauren M. Aiani, Alisa Norvelle, Kymberly N. Grantham, Kylie O'Laughlin, Joseph I. Terranova, Kyle J. Frantz, H. Elliott Albers and anonymous reviewers

(Borland et al. 2019b)

4.1 Abstract

Social reward is critical for social relationships, and yet we know little about the characteristics of social interactions that are rewarding or the neural mechanisms underlying that reward. Here, we investigate the sex-dependent role of oxytocin receptors within the ventral tegmental area (VTA) in mediating the magnitude and valence of social reward. Operant and classical conditioning tests were used to measure social reward associated with same-sex social interactions. The effects of oxytocin, selective oxytocin receptor agonists, antagonists and vehicle injected into the VTA on social reward was determined in male and female Syrian hamsters. The colocalization of FOS and oxytocin in sites that project to the VTA following social interaction was also determined. Females find same-sex social interactions more rewarding than males and activation of oxytocin receptors in the VTA are critical for social reward in females, as well as males. These studies provide support for the hypothesis that there is an inverted U relationship between the duration of social interaction and social reward, mediated by oxytocin; and that in females the dose-response relationship is initiated at lower doses compared with males. Same-sex social interaction is more rewarding in females than in males, and an inverted U relationship mediated by oxytocin may have a critical role in assigning positive and negative valence to social stimuli. Understanding these sex differences in social

reward processing may be essential for understanding the sex differences in the prevalence of many psychiatric disorders and the development of gender-specific treatments of neuropsychiatric disorders.

4.2 Introduction

Social reward plays a critical role in the formation and maintenance of almost all adaptive social relationships (Trezza et al., 2011). Indeed, the very definition of the word social includes the concept of seeking or enjoying the companionship of others, aligning with definitions of reward stimuli as those that elicit approach (although social interactions can be negative as well). Despite the fundamental importance of social reward in biological and psychological health (Suomi et al., 1971; Snyder-Mackler et al., 2016), comparatively little is known about the rewarding properties of social interactions compared to the wealth of information available on the rewarding properties of other stimuli (e.g., drugs of abuse). The relationships between the characteristics of social interactions and their rewarding properties are not well understood. For example, only recently has it been shown that there is an inverse relationship between the reward value of social interactions and the frequency of seeking those interactions (Borland et al., 2018) that is similar to the relationship between drug seeking and the reward value of drugs (e.g. cocaine dose) (Maldonado et al., 1993; Veeneman et al., 2012; Doherty et al., 2013). In other words, the duration of social interaction is analogous to the dose or concentration dependent effects seen with drugs of abuse. Recently, we have taken a heuristic approach to better understand social reward by developing the hypothesis that an inverted U describes the relationship between the dose (i.e. duration and/or intensity) of social interaction and the reward value of those interactions (Borland et al., 2019a), similar to the inverted U-shaped dose-response relationship between drug dose and reward value (Uhl et al., 2014). Initially, as the dose increases, reward value also rises, but only to a point. Once this peak is reached, increasing dose further drives down the reward value. Thus, as the duration or intensity of social

interactions increase, the rewarding properties of those interactions would be initially increased, and then ultimately reduced.

The neural mechanisms mediating social reward remain to be fully defined. Recent evidence, however, indicates that the mesolimbic dopamine system, a circuit implicated in the rewarding properties of various stimuli including food, sex and drugs of abuse, is also critical for the rewarding effects of social stimuli. Key elements in this circuit are dopamine (DA) neurons that project from the ventral tegmental area (VTA) to the nucleus accumbens (NAc). Recently, the neuropeptide oxytocin (OT), long recognized for its important role in many forms of social behavior (Caldwell and Albers, 2016), has been implicated in mediating social reward by its actions in the VTA and NAc. Inhibiting OT receptors (OTR) in the NAc in male mice (Dolen et al., 2013) or in the VTA in male hamsters (Song et al., 2016) or mice (Hung et al., 2017) significantly attenuates the rewarding properties of social interactions. Furthermore, OT fibers are in close apposition to DA neurons in the VTA that project to the NAc in male rodents (Melis et al., 2007); OTR expression occurs in dopamine-containing neurons in the VTA (Peris et al., 2017); and activation of OTRs in the caudal VTA leads to dopamine efflux in the NAc (Melis et al., 2007; Shahrokh et al., 2010). Taken together, these data indicate that activation of OTRs within the mesolimbic dopamine system play a critical role in mediating or encoding the rewarding properties of social interactions.

Remarkably, nearly everything we know about the rewarding properties of social interactions and their neurobiological underpinnings have come from studies in males. Although social interactions are rewarding in both males and females (Douglas et al., 2004), even the most basic questions about sex-dependent differences in the rewarding properties of social interactions have not been pursued. Here we report the first evidence in an animal model that females find

same-sex social interactions to be significantly more rewarding than males. These studies were conducted in Syrian hamsters, a species particularly well-suited for the preclinical study of behaviors that underlie psychiatric health and illness (Terranova et al., 2016), and are consistent with recent evidence in humans that women find positive social interactions with same-sex partners to be more rewarding than men do (Feng et al., 2015). Further, we test the overarching hypothesis that there is an inverted U-shaped relationship between duration of social interaction and social reward that is mediated by OT in both males and females, and that this dose-response relationship is initiated at lower doses in females than males (Borland et al., 2019a). Understanding the sex differences in the mechanisms of social reward is particularly important because deficits in social reward are linked with a variety of psychiatric disorders (McGregor and Bowen, 2012; Foulkes et al., 2015), many of which are sex-dependent in terms of prevalence and predispositions, e.g. autism spectrum disorder (Young and Pfaff, 2014).

4.3 Material and Methods

Subjects

Female and male Syrian hamsters (n=372, 120-140 g) were purchased from Charles River Laboratory (Wilmington, MA) at 11 weeks old, and were housed singly, in a humidity and temperature controlled (22^{o}C) vivarium. All animals were housed in solid-bottom Plexiglas cages (43 x 22 x 20 cm) containing corncob bedding and cotton nesting material (Neslets; Ancare, Bellmore, NY) in a reverse light-dark (LD) cycle (14L:10D; lights off at 13:00). Food and water were available *ad libitum*. Hamsters acclimated for 2 weeks before experiments. Females were examined daily to determine the stage of their estrous cycle by monitoring vaginal secretion. Males were also handled daily to control for any handling effects that occurred in

females. Hamsters were weighed just prior to their first behavioral test. All behavioral tests were performed under red light during the first 3 hours of the dark phase of the LD cycle. All procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and approved by the Georgia State University Institutional Animal Care and Use Committee.

Conditioned Place Preference (CPP) Conditioning

For a detailed description of the CPP apparatus see Song et al. (2016a). Hamsters were given two 15 min pretests, three pairs of 10 min conditioning sessions, and one 15 min post-test. To control for the estrous cycle, females were pretested on diestrus and then given two days off (proestrus and estrus), followed by three pairs of conditioning sessions on diestrus and proestrus and then given one day off (estrus), and finally they were given a posttest on diestrus (Figure 4.1A). Male subjects were yoked with females, so they experienced the same protocol timeline as the females. In the pretest, hamsters were tested for their initial preference of the white and black chambers by allowing them to explore the apparatus freely for 15 min. The pretest was repeated again 24h later and the average of the two pretests was used for analysis. Immediately before each conditioning session, drugs or saline were injected into the VTA; hamsters were then placed in the non-preferred chamber for social interactions (10 min) and in the empty preferred chamber alone (10 min) each day for 3 days. Controls that received no social interaction were placed alone in both non-preferred and preferred chamber. In other words, hamsters had one trial of social interaction and one trial alone each day. The time between these trials was 1h and trials were conducted in a counter-balanced order. To control for the possibility of drug treatments being either rewarding or aversive independent of social interactions, hamsters always

received the same drugs immediately prior to both conditioning sessions (social interaction and alone). Pilot experiments performed in our lab found that injections of OT into the VTA do not have an effect on chamber preference in male hamsters independent of social interactions (Song et al., 2016a). Subjects were paired with a smaller (90-110g) non-aggressive same-sex stimulus hamster during social conditioning sessions. Subjects encountered a novel stimulus hamster for each training session. Two days after the last conditioning, hamsters were tested again for their chamber preference in the posttest in the same way as in the pretests. Hamsters were not injected with drugs or saline during the pretest and the posttest. The apparatus was cleaned with 100% ethanol prior to every session. Initial preference for white or black chambers was balanced between different treatment groups.

Operant Social Preference (OSP) Conditioning

For a detailed description of the OSP apparatus see Borland et al. (2017). In brief, the OSP apparatus was constructed of clear acrylic (Custom Plastics, Decatur, GA, USA). The apparatus consisted of three chambers: a main chamber and two smaller adjacent chambers. Each small chamber is separated from the main chamber by a one-way vertical-swing door; smaller chambers can only be accessed from main chamber. Chamber doors were brushed with steel wool to create coarse texture, distinct from the rest of the apparatus, and the doors were perforated by circular holes to allow airflow. Operant conditioning sessions began with hamsters placed in a designated drop zone against the far wall of the main chamber in the OSP apparatus, equidistant from both small chambers. A smaller (100-120g) non-aggressive (group housed), same-sex stimulus hamster was confined to either the left or right smaller chamber (Figure 4.1B). Assignment of the stimulus hamsters to the left or right chamber was counter-balanced across

experimental subjects. Twenty sec after entry into either small chamber, the subject was returned to the drop zone in the main chamber. A new stimulus hamster was provided for each subject on each test day. Subjects were allowed to move throughout the apparatus, while stimulus hamsters were confined to one of the small chambers. An initial acquisition session lasted 10-30 minutes; each subject was required to enter the chamber holding the stimulus animal at least 3 times. All test sessions except the first acquisition session were 10 min in duration.

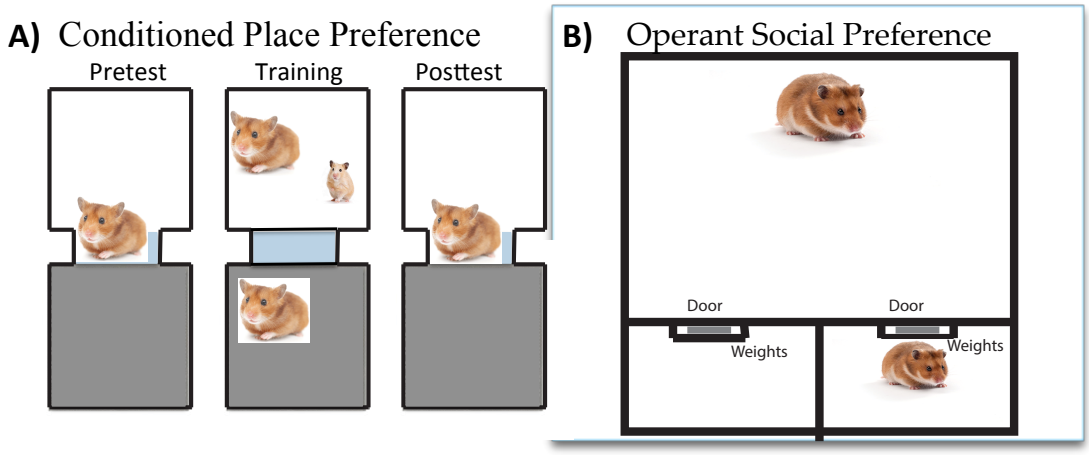


Figure 4.1: Social reward tests.

A) Conditioned Place Preference test: Female hamsters were pretested (15 minute free-run throughout the device) on the first and second days of diestrus (D1 & D2), followed by 2 days off on proestrus and estrus (P & E). Hamsters then had three consecutive days of paired conditioning sessions (10 minutes in preferred and non-preferred chambers) on D1, D2 and P, followed by 1 day off on E. Conditioning sessions consisted of being alone in initially preferred chamber and either social interaction with another same-sex stimulus hamster or being alone in the initially non-preferred chamber. In microinjection studies, subjects were injected with either saline, oxytocin or an oxytocin receptor agonist or antagonist into the caudal ventral tegmental area five minutes prior to conditioning sessions in both preferred and non-preferred chambers. Hamsters received a final posttest on D1. Testing in males was yoked to the testing in females.

B) Operant Social Preference test: An experimental hamster was placed in the main larger chamber and a stimulus hamster occupied one of the side chambers, separated by the vertical-swing door. The door allows visual, auditory and olfactory cues to enter the small chambers through the open top and holes in the door. All sessions were 10 minutes except for the pretesting session which was 15 minutes. Stimulus hamsters were always the same sex as the experimental hamster.

Behavior Scoring

All behavior tests were videorecorded (Panasonic-WVCP294) and analyzed using the Noldus Observer system (11.5, Leesburg, VA). An experimenter blind to the treatment groups scored each videotape. Time spent in the preferred and non-preferred chambers during pretest and posttest sessions were recorded using stopwatches. For social conditioning sessions, the following behaviors were also scored: duration of aggression, social investigation, submission (e.g. fleeing, avoidance), grooming, flank marking (a scent marking behavior), and non-social behavior. The number of attacks was scored as a point event during displays of aggression, and the number of flank markings was scored as a point event during non-social behavior. For operational definitions of these behaviors, see (Drickamer and Vandenbergh, 1973, Drickamer et al., 1973, Ferris et al., 1987, Albers and Rowland, 1989, Gray et al., 2015).

Stereotaxic Surgery

To prepare for intracerebral drug injections, hamsters were anesthetized with isoflurane (induced at 5% and maintained at 2-4%) and a 4 mm 26-gauge cannula was implanted unilaterally and aimed at the ventral tegmental area (VTA) (from bregma; anteroposterior (AP) -3.80 mm;

mediolateral (ML) +0.55 mm; dorsoventral (DV) -3.20 mm; 0^{o} angle). Guide cannulae were secured to the skull with screws, 11 mm wound clips and dental adhesive. Dummy caps were inserted to prevent clogging. Previous studies investigating the effects of OT in the VTA on social reward have also used unilateral injections (Song et al., 2016a, Hung et al., 2017). All hamsters were injected subcutaneously with the anti-inflammatory agent ketofen (5mg/kg) and allowed to recover for at least 4-6 days prior to behavioral testing.

Drug Treatment

Microinjections were administered using a 12mm, 32-gauge needle attached to a 1 µl Hamilton syringe that extended an additional 4.2 mm beyond cannula to a final depth of 7.4 mm below skull surface. Approximately 5 min after microinjection, hamsters were tested in the CPP apparatus. The drugs used were OT (Bachem, CA, USA) dissolved in sterile saline to a final concentration of 0.9µM and 9µM; [Thr4,Gly7]OT a highly selective OTR agonist (TGOT) (Manning et al., 2012) (gift from Dr. Maurice Manning) dissolved in sterile saline to a final concentration of 23µM; and desGly-NH_2-d$(CH_2)_5$[D-Tyr2,Thr4]OVT a highly selective OTR antagonist (OTA) (Manning et al., 2012) (gift from Dr. Maurice Manning) dissolved in sterile saline to a final concentration of 90µM. Drugs were delivered in a volume of 200 nl at a rate of 0.400µl min^{-1} using an infusion pump (Harvard Apparatus). The needle was left in place for an additional 30 seconds to allow diffusion away from the tip of the injection cannula. The concentrations of all drugs administered were based on the concentrations used in previous studies that were found effective in altering social reward behavior in hamsters (Song et al., 2014, Song et al., 2016a, Song et al., 2016b).

Histology

Within 24 hr of the final behavioral test, hamsters were euthanized with a lethal dose of sodium pentobarbital (0.2 ml, i.p., Henry Schein Animal Health, Dublin, OH) and 200 nl of India ink was microinjected through the guide cannula to mark the injection site. Brains were extracted and submerged in 10% formalin for at least 24 hr at 4^{o} C. Brains were sectioned at 40μm with a cryostat, mounted on superfrost plus slides, and stained with neutral red. The sites of injection were considered to be accurate if ink was seen within the caudal VTA, referenced to the hamster stereotaxic atlas (Morin and Wood, 2001). Misses were excluded from statistical analysis.

Immunocytochemistry

To investigate neural activation during social behavior, hamsters were paired with a smaller, group-housed, sex-matched stimulus hamster in a neutral arena for 10 min, and social behavior was quantified during the social interactions. Sixty minutes after the start of behavior testing, hamsters were euthanized as described earlier, transcardinally perfused, and tissue processed as previously described (Terranova et al., 2016). Brains were sectioned at 40μm in the coronal plane on a cryostat and stored in a cryoprotectant solution (500 mL PBS, 300 g sucrose, 10 g polyvinyl pyrrolidone, 300 mL ethylene glycol) until immunofluorescent processing. Sections containing the hypothalamic paraventricular and supraoptic nuclei were processed. All immunofluorescent procedures were conducted at room temperature. Sections were washed in PBS five times for 5 min and blocked in 10% normal donkey serum (NDS) with 0.4% Triton X-100 and 3% H_2O_2 in PBS for 1 h. Sections were then incubated overnight in an antibody solution (ABS: 0.4% of Triton X-100 and 2% NDS in PBS) for rabbit anti-OT T-4084: (Peninsula Laboratories) (1/5,000) and mouse anti-c-Fos (ab208942: abcam) (1/500), a marker of

neural activation. Sections were washed in PBS five times for 5 min and incubated in darkness for 2 h in ABS containing secondary antibodies. Alexa Fluor 488 conjugated-donkey anti-rabbit IgG and 594 conjugated-donkey anti-mouse IgG (1/250; Jackson Immunoresearch). All tissue was washed in 100 mM cupric sulfate in 50 mM ammonium acetate (pH: 5) for 5 min and then washed in TBS (900 ml dH_2O, 48.44 g Trizma base, 15.76 g Trizma HCl, 9.0 g sodium chloride, pinch sodium azide, pH: 7.4) three times for 5 min. Tissue was then washed in PBS six times for 5 min. Finally, tissue was mounted onto Colorfrost Plus Microscope Slides (12-550-17; Fisher Scientific) in PBS, rinsed with dH_2O, and coverslipped with Vectashield Hard Set Mounting Medium for Fluoescence (H1400; Vector Laboratories).

Confocal Microscopy and Quantification

Digital images were acquired using Zeiss LSM 720 confocal microscope at 20x magnification and ZEN 2012 software. Z stack images at 2.0 μm intervals were obtained, and 3-5 representative images of entire regions from each subject were quantified and averaged. Overall adjustments to brightness were applied evenly to channels of all images to maximize clarity. The "Cell Counting" plugin in ImageJ was used for quantification of cells staining for c-Fos and co-localized with OT. Digital zooming was used to confirm colocalization. Cell activation was determined by quantifying cells containing staining for c-Fos, co-localization with OT. Images in figures are maximum intensity projection images.

Statistical Analysis

Data were analyzed using SPSS software (23.0, SAS Institute, 1990) for Windows. All data were examined to determine if the assumptions of parametric analyses were met. When

assumptions were violated, data were square-root transformed. Dependending on the experimental design, independent t-tests or mixed repeated measures ANOVAs were performed to investigate effects of sex, drug treatment, chamber condition and test session on either number of entries into chambers, time spent in chambers, social behavior or neuron cell count. All tests were two-tailed, and results considered statistically significant if $p \leq 0.05$. All data are presented as mean ± standard error of the mean.

4.4 Results

Experiment 1: Sex differences in the rewarding properties of social interaction

In the following experiments, we investigated the rewarding properties of same-sex social interactions in male and female hamsters. Before beginning these experiments, however, we examined whether there were differences in the rewarding effects of social interactions in females at different stages of the estrous cycle, using an operant social preference test. Females entered chambers containing female stimulus hamsters significantly more often than empty chambers ($p<0.001$, $F(1,21) = 46.241$), and no significant differences were observed over the days of the estrous cycle ($p=0.665$, $F(3,21) = 0.533$) (Figure 4.2A, B). Although the rewarding effects of social interaction thus appeared not to change over the estrous cycle, females did display several expected estrous cycle-dependent changes in social behavior, e.g. aggression and social communication (Figure 4.2C, D).

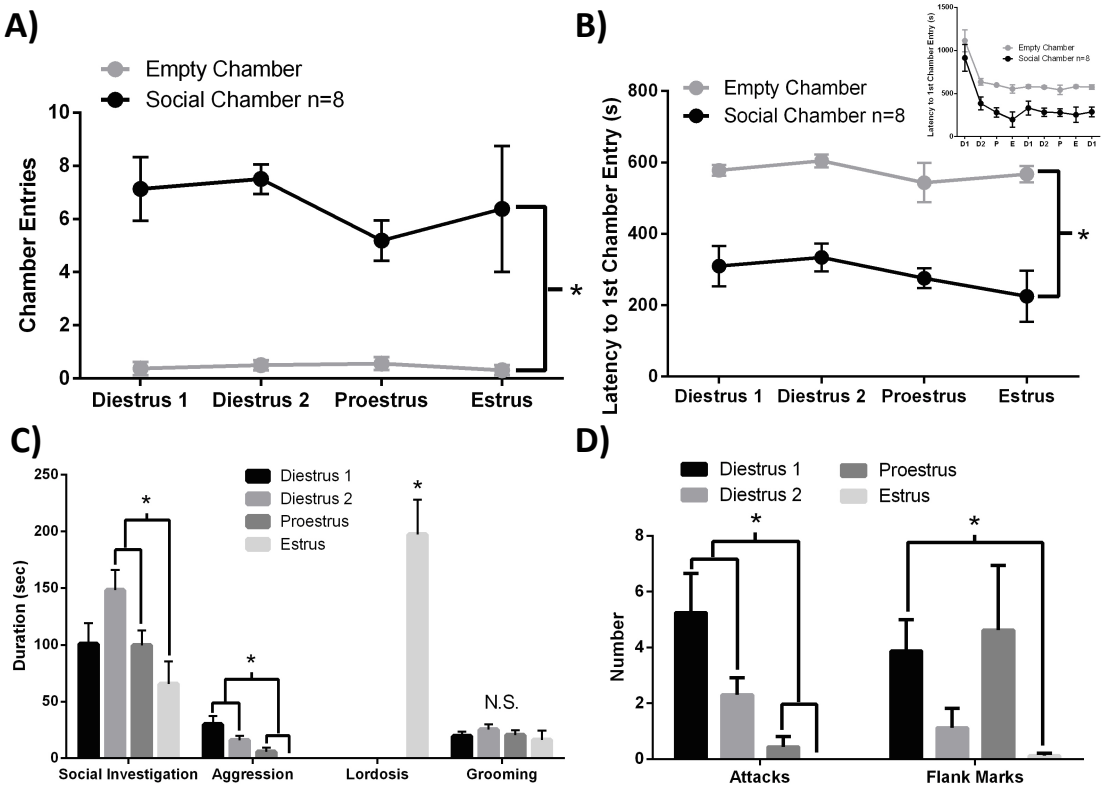


Figure 4.2: Social preference and social behavior in female hamsters across the estrous cycle measured using the Operant Social Preference test.

A) There were no significant differences in the number of entries into the chambers across the estrous cycle ($p=0.665$, $F(3,21) = 0.533$), but hamsters entered into chambers containing stimulus hamsters significantly more than empty chambers ($p<0.001$, $F(1,21) = 46.241$). B) There were no significant differences in the latency to enter the chambers across the estrous cycle ($p=0.403$, $F(3,21) = 1.022$), but hamsters had a significantly shorter latency to enter chambers containing stimulus hamsters than empty chambers ($p<0.001$, $F(1,21) = 55.092$). Latency scores across all 9 test days (inset). C) The duration of social investigation was significantly shorter on estrus than on diestrus 2 ($p=0.044$) or proestrus ($p=0.048$: $p=0.036$, $F(3,21) = 3.431$). The duration of aggression was significantly lower on proestrus ($p=0.019$) and estrus ($p<0.001$) than diestrus ($p<0.001$, $F(3,21) = 14.327$). The duration of lordosis was significantly longer on estrus than

any other day of the estrous cycle ($p<0.001$, $F(3,21) = 41.710$). There were no significant differences in the duration of grooming over the estrous cycle ($p=0.675$, $F(3,21) = 0.518$). D) The number of attacks were significantly lower on proestrus ($p=0.005$) and estrus ($p<0.001$) compared to diestrus ($p<0.001$, $F(3,21) = 18.206$). The number of flank marks were significantly lower on estrus compared to diestrus 1 ($p=0.011$; $p=0.062$, $F(3,21) = 2.857$). There was no main effect of estrous cycle on chamber preference score ($p=0.581$, $F(3,21) = 0.668$) (data not shown). (* indicates significant differences between groups, $p<0.005$)

To test for sex differences in the rewarding properties of social interactions, the number of entries into a chamber containing a same-sex hamster was compared in males and females. Both males and females entered chambers containing same-sex hamsters significantly more than they entered empty chambers ($p<0.001$, $F(1,140) = 153.798$; Figure 4.3A). Females, however, had a significantly greater social preference score (number of entries into the social chambers minus number of entries into the empty chambers) than males ($p=0.003$, $F(1,14) = 12.615$; Figure 4.3C). Indeed, the social preference score in males was 38.4% lower than in females.

Additional evidence that females find same-sex social interactions to be more rewarding than males was revealed using the conditioned place preference test. As expected, both males and females spent significantly more time in the chamber where the stimulus hamster had been paired, compared to the empty chamber ($p<0.001$, $F(1,17) = 87.919$; Figure 4.3B). Females, however, displayed a significantly greater change in the social chamber preference score compared to males ($p=0.004$, $F(1,17) = 11.050$; Figure 4.3D). The social preference score was 57.2% lower in males than in females.

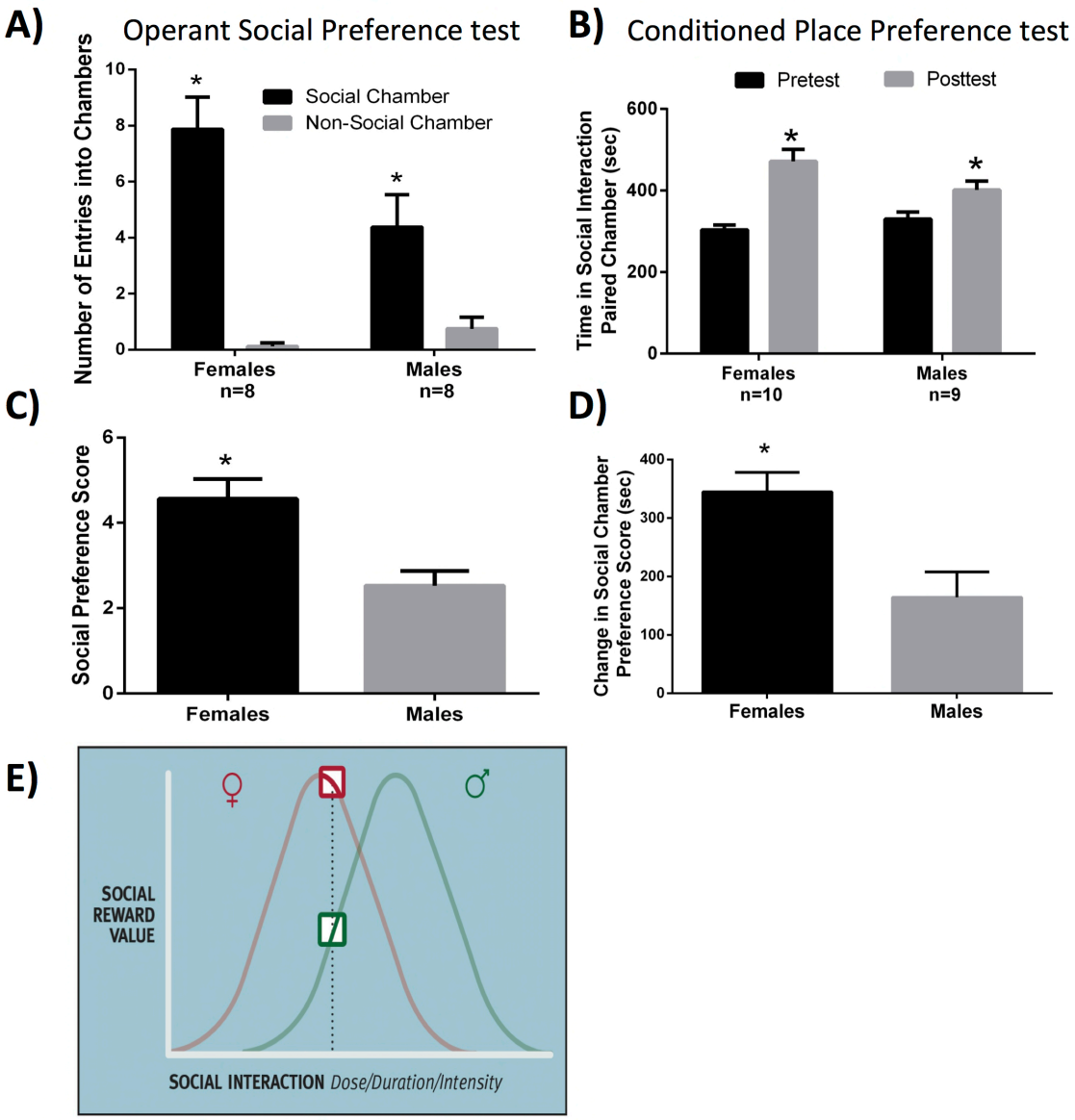


Figure 4.3: Sex difference in the rewarding properties of same-sex social interaction.

A) Both males and females made more entries into the chambers containing same-sex stimulus hamsters compared to empty chambers in the operant social preference test. B) Both males and females displayed a significant increase in the time spent in the chambers associated with same-sex social interactions during the post-test compared to the pre-test in the conditioned place preference test. C) Females had a significantly higher social preference score (number of entries into the social chambers minus number of entries into the empty chambers) in the operant social

preference test than males. D) Females had a greater social preference score (time spent in the social interaction chambers minus the time spent in the no social interaction chamber) in the conditioned place preference test than males. E) Illustration of the inverted U hypothesis of the relationship between the dose/duration/intensity of social interactions and their reward value in males and females. In females the inverted U relationship is shifted to the left relative to males because social reward is initiated at lower doses of social interaction in females than in males. As a result, social interactions that are maximally rewarding in females are only suboptimally rewarding in males. (* indicates significant difference between groups, $p<0.05$). Error bars S.E.M.

To determine whether the sex differences in social place preference might result from sex differences in the characteristics of the same-sex agonistic encounters, we quantified the social behaviors that occurred during these interactions. No sex differences were observed in the duration of social investigation, aggression, grooming, or in the number of attacks or flank marks ($p>0.05$; Figure 4.4).

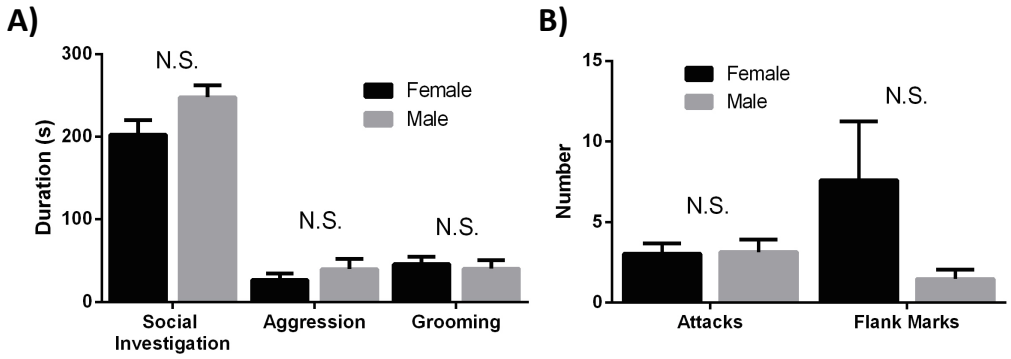


Figure 4.4: Social behavior in males and females during social conditioning sessions in the Conditioned Place Preference test

Panel A: There were no sex differences in the duration of social investigation ($t(17) = -1.940$, $p=0.069$), aggression ($t(17) = -0.911$, $p=0.375$) or grooming ($t(17) = 0.403$, $p=0.692$) during the social conditioning sessions. Panel B: There were no sex differences in the number of attacks ($t(17) = -0.115$, $p=0.910$) or flank marks ($t(17) = 1.569$, $p=0.086$) in females and males.

Experiment 2: OTR activation in the VTA is necessary for social reward in both males and females

We next investigated whether activation of OTRs within the VTA is necessary for social interactions to be rewarding in males and females, using the conditioned place preference test. A highly selective OTR antagonist or vehicle was injected into the caudal VTA five minutes prior to each of three social interaction conditioning sessions. The OTR antagonist significantly decreased the time spent in the chamber where the same-sex social interaction occurred in both males ($p=0.020$) and females ($p=0.003$), compared to vehicle injected controls ($p=0.001$, $F(1,36) = 14.556$; Figure 4.5B). Likewise, for the preference score, injections of the OTR antagonist decreased preference for the chamber where social interactions occurred in both males ($p=0.027$) and females ($p=0.012$), compared to controls ($p=0.002$, $F(1,36) = 11.221$; Figure 4.5C). The effect size for the OTR antagonist to reduce the time spent in the social-paired chamber and social preference score was 0.306 and 0.254 respectively. Finally, as in the previous experiments, females injected with saline spent more time in the social-paired chamber compared to males injected with saline ($p=0.024$; females 176.2sec +/-29.3; males 104.1sec +/-22.7; Figure 4.5B) and differences in the social chamber preference score just missed significance ($p=0.081$; females 341.50sec +/-53.65; males 228.51sec +/-54.94; Figure 4.5C).

Representative sites of injection are in Figure 10; analysis excluded one male with the site of OTR antagonist injection outside the caudal VTA.

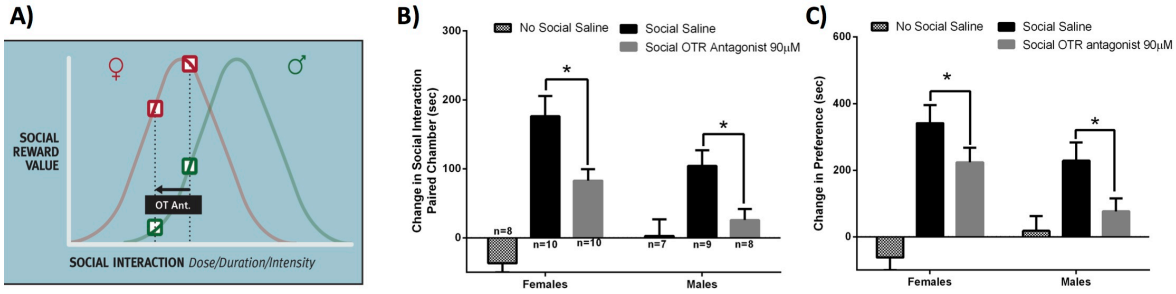


Figure 4.5: Effects of inhibiting oxytocin receptor (OTR) activation in the caudal ventral tegmental area (VTA) with a highly selective OTR antagonist (Ant.) on the rewarding properties of social interaction in the conditioned place preference test.

Males and females experienced same-sex social interaction in their initially non-preferred chamber and received injections of either saline or a highly selective OTR antagonist (90µM) into the caudal VTA five minutes prior to social conditioning sessions. A) The inverted U hypothesis predicts that antagonism of OTR activation reduces social reward in both males and females. B) Male and female controls injected with saline in the VTA but not paired with other hamsters (i.e., No Social Saline) displayed no change in the time spent in the chambers during the post-test. In both males and females injected with saline prior to social interactions (Social Saline) there was an increase in the time spent in the social interaction paired chambers during the post-test. In both males and females, injection of the OTR antagonist into the VTA (Social OTR Antagonist) decreased the time spent in the social interaction paired chambers compared to saline injected controls during the post-test. C) Male and female controls (i.e., No Social Saline) displayed no change in the social chamber preference score. In both males and females injected with saline prior to social interactions (Social Saline) there was an increase in the social chamber preference score. In both males and females, injections of the OTR antagonist into the VTA

(Social OTR Antagonist) decreased the social chamber preference score compared to saline injected controls. (* indicates significant difference between groups, $p<0.05$)

Again, we examined whether there were between group differences in the quality of social behavior observed during social pairings. The OTR antagonist in the VTA decreased the duration of social investigation in both males and females ($p=0.010$, $F(1,33) = 7.465$; Figure 4.6). No significant effects, nor sex differences were observed in the other behaviors measured ($p>0.05$).

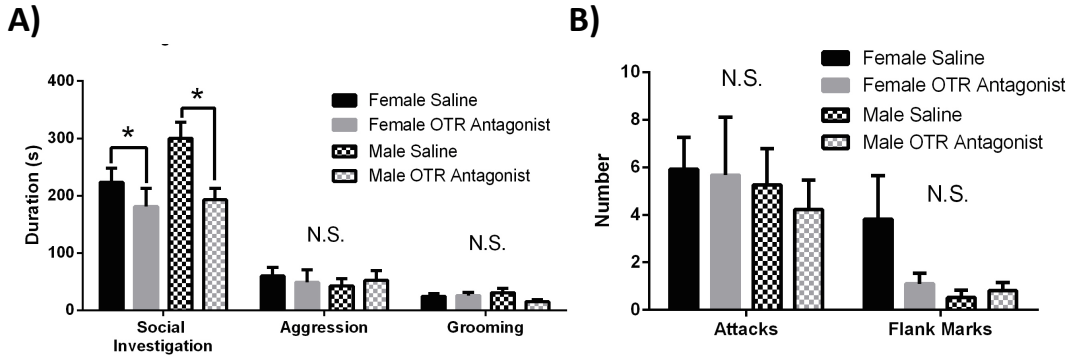


Figure 4.6: Sex differences in the effects of the oxytocin receptor (OTR) antagonist injected into the caudal ventral tegmental area (VTA) during social conditioning sessions in the Conditioned Place Preference test.

A) The duration of social investigation was significantly lower ($p=0.010$, $F(1,37) = 7.465$) in both females and males injected with the OTR antagonist compared to subjects injected with saline. There was no effect of sex ($p=0.115$, $F(1,37) = 2.626$) on the duration of social investigation. No significant differences were observed in the duration of aggression ($p=0.975$, $F(1,37) = 0.001$) and the duration of grooming ($p=0.200$, $F(1,37) = 1.707$) between subjects injected with the OTR antagonist and subjects injected with saline. Similarly, there were no differences between males and females in the duration of aggression ($p=0.685$, $F(1,37) = 0.167$)

or the duration of grooming (p=0.718, F(1,37) = 0.133). B) There was also no effect of OTR antagonist injection (p=0.720, F(1,37) = 0.131; p=0.257, F(1,37) = 1.328) or sex (p=0.558, F(1,37) = 0.351; p=0.098, F(1,37) = 2.907) on the number of attacks or flank marks respectively.

Experiment 3: Sex differences in the effects of intra-VTA OT on social reward.

Next, we tested the hypothesis that an inverted U-shaped relationship exists between the dose of social interaction and social reward, mediated by OT in both males and females, but that this dose-response relationship is initiated at lower doses in females than males, i.e. shifted leftward in females. In the first experiment, we tested the effects of 9μM OT injected into the VTA on the rewarding properties of three social interaction sessions (10 min per session) in males and females. This dose of OT was chosen because we have previously shown that it significantly increases the rewarding effects of social interactions in males. We hypothesized that this "dose" of social interaction (3 X 10 min sessions) is on the ascending slope of the inverted U-shaped relationship in males. In contrast, because the inverted U-shaped relationship between the dose of social interaction and social reward is initiated at lower doses in females, we predicted that injection of this same 9μM concentration of OT into the VTA of females would significantly reduce the rewarding effects of the three sessions of social interactions by driving them into the downward slope of the inverted U (Figure 4.7A). As predicted, in females, 9μM OT injected into the VTA prior to conditioning sessions decreased the time spent in the social-paired chamber (p=0.001, OT n=14, 54.42sec +/-20.6; saline n=12, 157.6sec +/-25.0) and decreased the social chamber preference score (p<0.001, OT n=14, 110.0sec +/-44.2; saline n=12, 321.5sec +/-48.1) compared to saline controls (Figure 4.7 B, C). In males, however, 9μM OT in the VTA increased the time spent in the social-paired chamber (p=0.020, OT n=11,

149.0sec +/-18.4; saline n=14, 80.2sec +/-20.1) and the change in social chamber preference score just missed significance ($p=0.056$, OT n=11, 277.3sec +/-34.9; saline n=14, 164.1sec +/-40.6), compared to saline controls (Figure 4.7 B, C). As in the previous studies, females displayed a greater change in the time spent in the social-paired chamber than males ($p=0.008$) and a greater change in social chamber preference score ($p=0.007$). Again, neither sex nor OT exerted effects on aggression or social communication, although grooming differed by sex ($p<0.001$, $(F1,47) = 23.602$) and OT decreased the duration of social investigation in females ($p=0.006$, OT n=14, 288.7sec +/-22.3; saline n=12, 212.5sec +/-13.8), but not males ($p=0.404$, OT n=11, 176.4sec +/-21.5; saline n=14, 153.5sec +/-18.4) (Figure 4.8). Eleven females and 10 males were injected with 9μM OT outside the caudal VTA and were excluded from statistical analysis. Results from these hamsters injected with OT outside the caudal VTA (i.e., misses; Figure 4.10) were similar to the results obtained from saline treated controls ($p=1.000$ for females; $p=0.766$ for males; data not shown).

Next, we reduced the concentration of OT injected into the VTA prior to conditioning sessions to 0.9μM. Here, the inverted U-shaped hypothesis predicted that social reward would decrease a small amount in females, increase a small amount in males, but that the absolute amount of social reward would be similar in males and females (Figure 4.7D). Although there were no significant differences across groups, trends indicated that injections of 0.9μM OT into the VTA slightly reduced both measures of social reward in females ($p=0.171$, $t(19) = 1.422$; time in social-paired chamber: $p=0.388$, $t(19) = 0.884$; social chamber preference score) and increased both measures in males ($p=0.084$, $t(18) = -1.830$; time in social-paired chamber: $p=0.115$, $t(18) = -1.654$; social chamber preference score) (Figure 4.7 E, F). In addition, the change in the time spent in the chambers associated with social interaction ($p=0.976$, $t(20) = -$

0.305) and the change in the preference scores (p=0.764, t(20) = -0.030) were almost identical in males and females. Investigating the effects of additional concentrations of OT was not practical, as concentrations of OT below 0.9μM are unlikely to have behavioral effects and the injection of concentrations of OT above 9.0μM into the VTA induces behavioral effects that compromise the measurement of social reward. In this experiment, two females and one male were excluded from statistical analysis due to injection sites outside the caudal VTA.

To further test the inverted U hypothesis, we manipulated the dose of social interaction by reducing the number of 10 min social interaction sessions from three to one. Here, we predicted that reducing the dose of social interaction would place females on the ascending slope of the inverted U-shaped relationship and as a result injection of 9μM OT would significantly *increase* social reward instead of *decreasing* as it did when the dose of social interaction was higher. OT injected into the VTA prior to the social interaction session increased the time spent in the chamber where the social interaction occurred (p=0.048, t(12)=2.205; Figure 4.7H) and the social chamber preference score (p=0.044, t(12)=2.247; Figure 4.7I) compared to saline treated female hamsters. No subjects were excluded due to injection sites outside the caudal VTA (Figure 4.10).

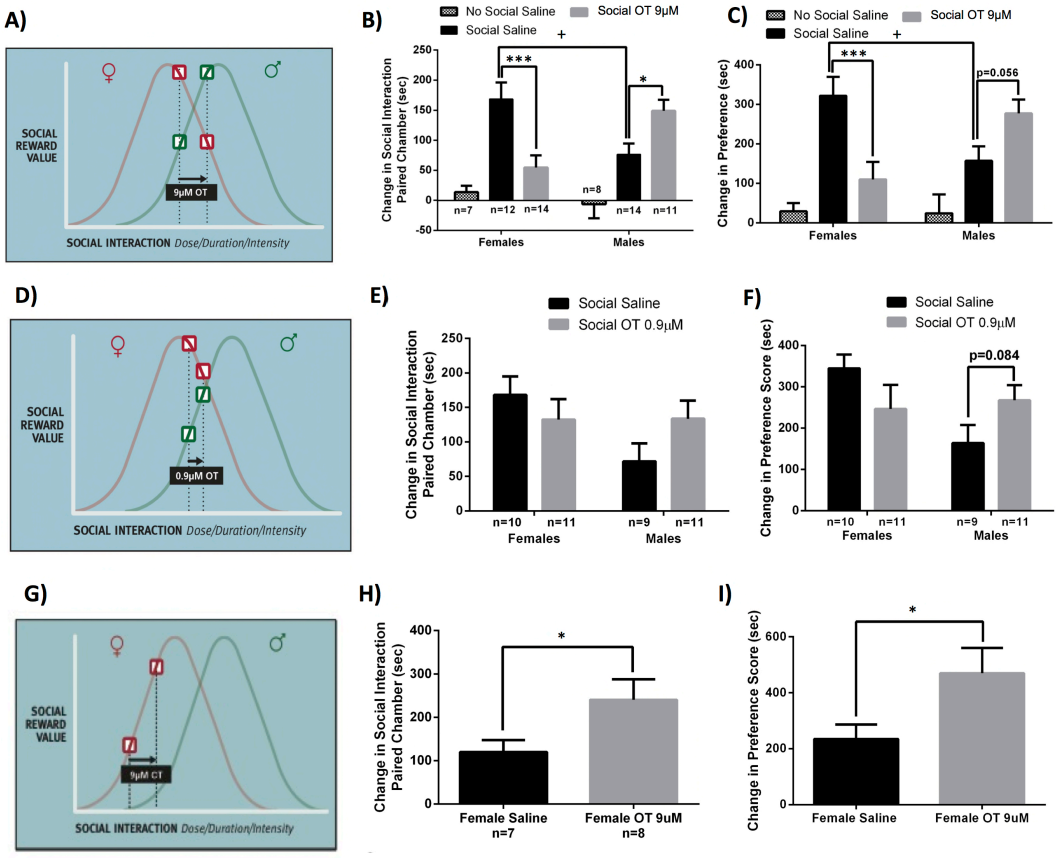


Figure 4.7: The effects of oxytocin (OT) injected into the caudal ventral tegmental area (VTA) on social reward.

(A-C) Males and females were injected with either 9μM OT or saline into the VTA five minutes prior to each of the three social interaction conditioning sessions in the conditioned place preference test. A) The inverted U hypothesis predicts that injection of 9μM OT will *decrease* social reward in females but *increase* social reward in males. B) As predicted, OT injected into the VTA *decreased* the change in the time spent in the social interaction paired chamber compared to saline controls during the post-test in females (Social Oxytocin); but in males, OT *increased* the change in the time spent in the social interaction paired chamber compared to saline controls. C) In females, OT injected into the VTA *decreased* the change in the social chamber preference score compared to saline controls (Social Oxytocin); but in males, injection

of OT *increased* in the change in the social chamber preference score compared to saline controls just missing statistical significance. (D-F) The effects of oxytocin (OT) (0.9μM) injected into the VTA on social reward in males and females. D) The inverted U hypothesis predicts that injection of 0.9μM OT into the VTA will *decrease* social reward a small amount in females and *increase* social reward a small amount in males, but that the absolute amount of social reward would be similar in males and females. E) In both males and females, OT injected into the VTA (Social OT) had no statistically significant effect on the change in the time spent in the social interaction paired chamber during the post-test compared to controls (Social Saline). There was, however, a trend for OT to decrease the time spent in the social interaction paired chamber in the post-test compared to controls in females and for OT to increase the time spent in the social interaction paired chamber compared to controls in males. F) In females, there was a trend for OT injected into the VTA to decrease the change in the social chamber preference score compared controls and in males, the increase in the change in the social chamber preference score compared to controls just missed significance. (G-I) The effects of oxytocin (OT) injected into the VTA on social reward in females given only a single social interaction conditioning session. G) The inverted U hypothesis predicts that reducing the dose of social interaction would place females on the ascending slope of the inverted U-shaped relationship and, as a result, injection of 9μM OT would *increase* social reward instead of *decreasing* it as it did when the dose of social interaction was higher. H) OT injected into the VTA increased the change in the time spent in the social interaction paired chamber during the post-test compared to saline injections. I) OT increased the change in the social preference score compared to saline injections. (* indicates significant difference between drug groups, * for $p<0.05$, ** for $p<0.01$, *** for $p<0.001$; + indicates significant difference between sexes, $p<0.05$).

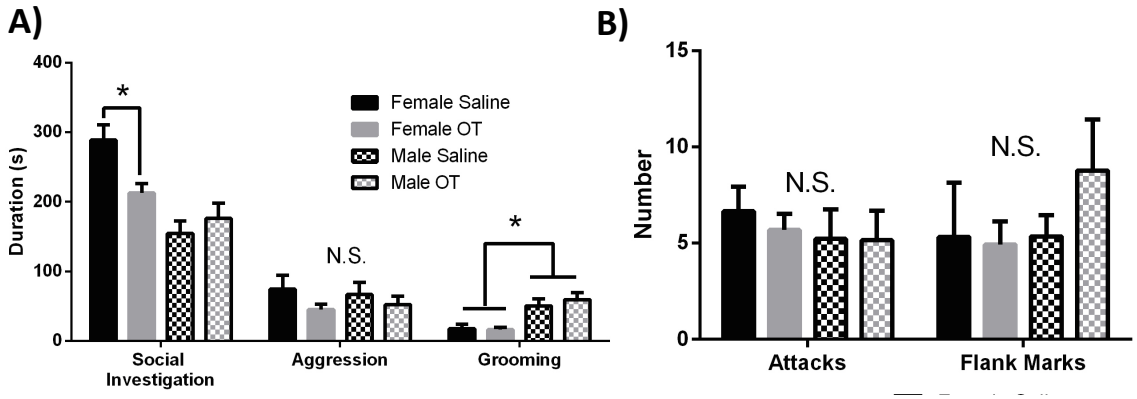


Figure 4.8: Sex differences in the effects of oxytocin (OT) injected into the caudal ventral tegmental area (VTA) during social conditioning sessions in the Conditioned Place Preference test.

A) The duration of social investigation was significantly lower in females injected with OT compared to females injected with saline ($p=0.006$). No significant differences were observed in the duration of social investigation between males injected with OT and males injected with saline ($p=0.426$; interaction: $p=0.012$, $F(1,47) = 6.813$). There were no differences between females and males ($p=0.997$, $F(1,47) = 0.000$) nor saline and oxytocin treated subjects ($p=0.144$, $F(1,47) = 2.205$) in the duration of aggression. The duration of grooming was higher in males compared to females ($p<0.001$, $(F1,47) = 23.602$) independent of OT treatment ($p=0.648$, $F(1,47) = 0.211$). B) There was no effect of OT injection ($p=0.675$, $F(1,47) = 0.178$; $p=0.903$, $F(1,47) = 0.015$) or sex ($p=0.474$, $F(1,47) = 0.521$; $p=0.875$, $F(1,47) = 0.025$) on the number of attacks or flank marks respectively.

Experiment 4: The effects of OT on social reward in males and females are mediated by the activation of OTRs, not vasopressin receptors.

The effectiveness of a highly selective OTR antagonist in the previous experiments supports the hypothesis that OTRs play an important role in mediating social reward in the VTA. However, it is unclear if the sex specific effects of OT in the VTA on social reward are specifically mediated by activation of OTRs. Further support for the role of OTRs in mediating the effects of OT was provided in the next experiment in which a highly selective OTR agonist was injected into the VTA. An interaction of sex and drug was observed for both the time spent in the social interaction associated chamber ($p<0.001$, $F(1,35) = 17.553$) and social chamber preference score ($p<0.001$, $F(1,35) = 16.664$). As observed above with OT, in females the OTR agonist injected into the VTA decreased the time spent in the social interaction associated chamber ($p<0.001$, OTR agonist n=10, 33.8sec +/-25.5; saline n=10 176.2sec +/-29.3) and decreased the social chamber preference score ($p<0.001$, OTR agonist n=10, 48.8sec +/-52.3; saline n=10, 341.5sec +/-53.6) compared to saline controls. In males, a trend for the OTR agonist injected into the VTA to increase the time spent in the social-paired chamber ($p=0.062$, OTR agonist n=8, 166.7sec +/-23.6; saline n=9, 104.1sec +/-22.7) and social chamber preference score was observed, compared to saline controls ($p=0.124$, OTR agonist n=8, 333.4sec +/-45.9; saline n=9, 228.5 +/-54.9; Figure 4.9 A, B). The sites of injection in four females and nine males were outside the caudal VTA (Figure 4.10), and results from these hamsters were similar to saline controls (data not shown).

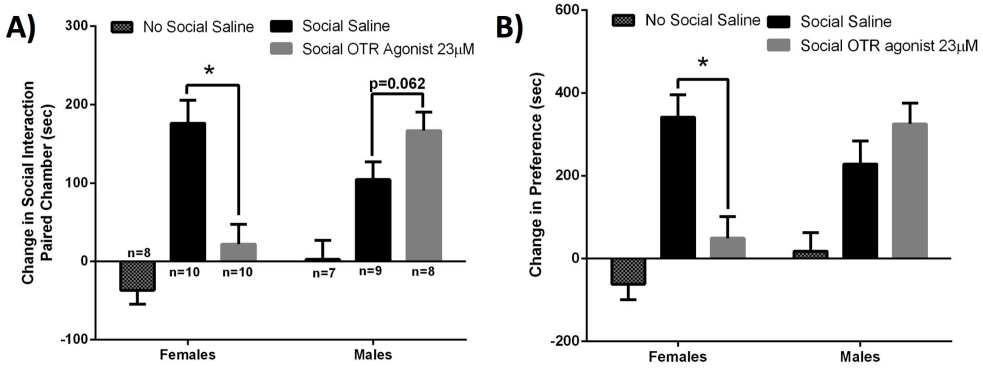
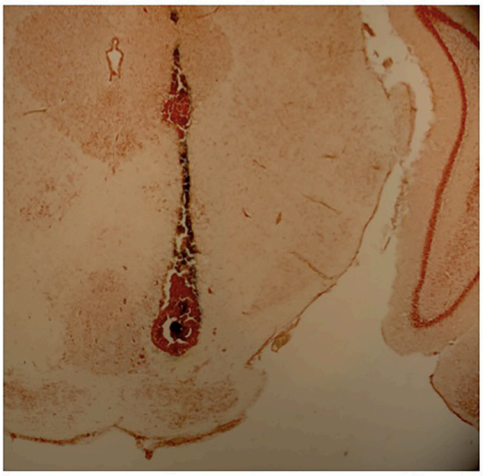


Figure 4.9: The effects of injection of a highly selective oxytocin receptor (OTR) agonist into the caudal ventral tegmental area (VTA) supports the hypothesis that social reward is mediated by the activation of OT receptors (OTRs) and not vasopressin receptors in males and females.

Males and females were injected with either the OTR agonist (23μM) or saline into the VTA five minutes prior to each of the three social interaction conditioning sessions in the conditioned place preference test. A) Male and female controls injected with saline in the VTA but not paired with other hamsters (No Social Saline) displayed no change in the time spent in the chambers during the post-test. In both males and females injected with saline prior to social interactions (Social Saline) there was an increase in the time spent in the social interaction paired chambers during the post-test. Injections of the OTR agonist (Social OTR agonist) significantly decreased the time spent in the social interaction paired chambers during the post-test compared to saline controls in females, but in males, the OTR agonist increased the time spent in the social interaction paired chamber during the post-test although this difference just missed significance. B) Male and female controls (No Social Saline) displayed no change in the social chamber preference score. Both males and females injected with saline showed an increase in the social chamber preference score. Injections of the OTR agonist (Social OTR agonist) decreased the social chamber preference score compared to saline controls in females, but for males, the OTR agonist had no effect on social chamber preference score.

A)



B)

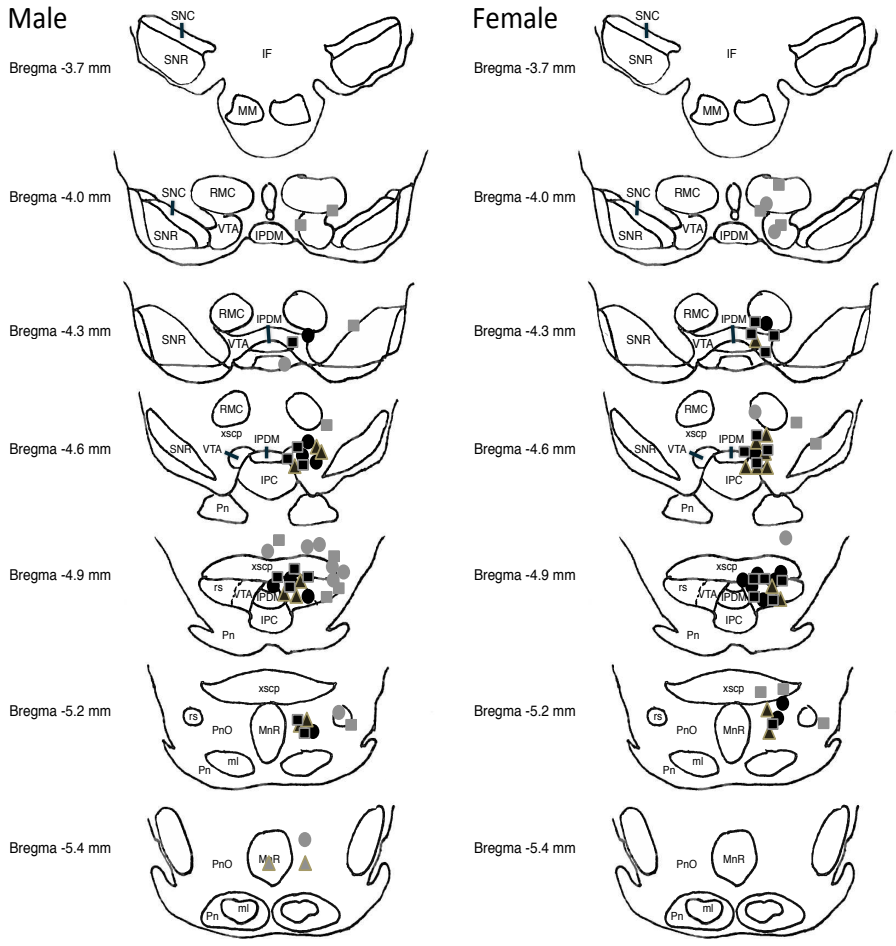


Figure 4.10: Microinjection histology.

A) Representative image of injection tract. B) Histology of representative injection sites of oxytocin (square), OTR agonist (circle) and OTR antagonist (triangle) in the conditioned place preference test experiments. Subjects with ink found within the caudal ventral tegmental area (VTA) were classified as hits (black), while subjects with ink found outside the caudal VTA were classified as misses (grey). Data obtained in subjects with misses was excluded from statistical analysis.

Experiment 5: Social interaction activates OT-ir neurons in the paraventricular nucleus (PVN) and the supraoptic nucleus (SON) of males and females.

The preceding experiments suggest that activation of OTRs in the VTA exerts sex-dependent effects on social reward. It is also possible, however, that sex differences in the extracellular levels OT within the VTA mediate sex differences in the rewarding properties of social interactions. One source of increased extracellular OT in the VTA may be the activation of hypothalamic magnocellular neurons. Indeed same-sex social interactions activate magnocellular neurons containing OT in the PVN of male mice. To determine if sex differences in endogenous activation of OT-containing neurons occur during social interaction, we quantified the co-localization of c-Fos and OT in the PVN (Figures 4.11A-D) and SON (Figures 4.11E-H) in male and female hamsters following a 10-min social interaction. Social interaction was associated with more neurons co-localized with OT and c-Fos in the PVN of both males and females ($p=0.037$, $F(1,46) = 4.593$), compared to isolated controls (Figure 4.11D). Likewise, social interaction appeared to increase activation of OT-containing neurons in the SON of males and females ($p<0.001$, $F(1,47) = 23.182$) (Figure 4.11H). Interestingly, a decrease in the total number of OT-containing neurons occurred in the PVN ($p=0.023$, $F(1,28) = 5.745$), but not the

SON (p=0.989, F(1,28) = 0.000). No sex differences in activation of OT-containing neurons occurred in either the PVN (p=0.217, F(1,46) = 1.564) nor SON (p=0.464, F(1,47) = 0.544). No sex differences in the expression of social behavior (duration of social investigation, aggression, grooming, and frequency of flank marks and attacks) were observed (Figure 4.12).

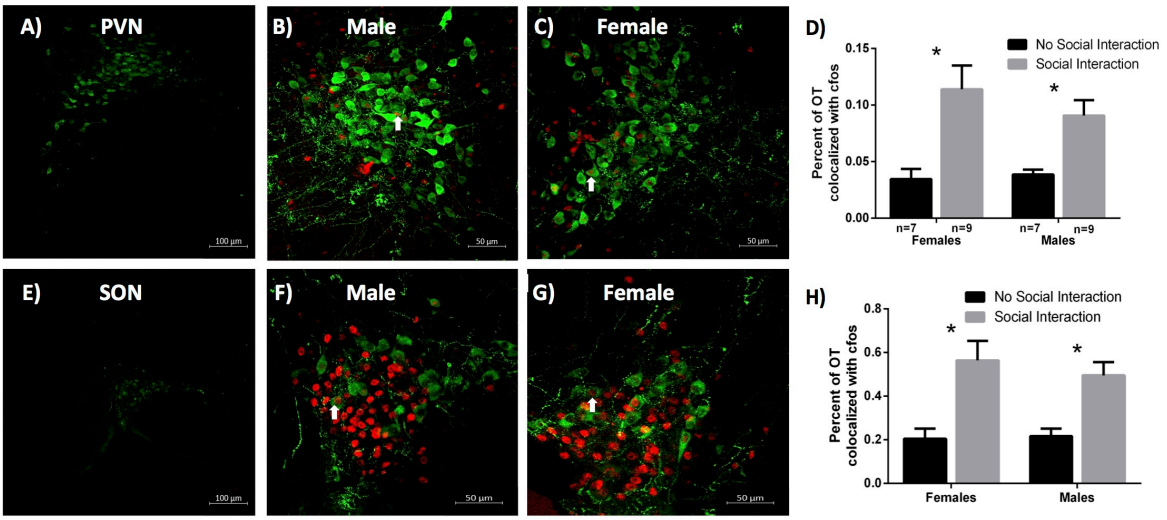


Figure 4.11: Social interaction activates oxytocin (OT) immunoreactive (ir) neurons in the paraventricular nucleus (PVN) and the supraoptic nucleus (SON) of the hypothalamus in males and females.

A) Representative PVN image, B) PVN of a male hamster, C) PVN of a female hamster. D) Both males and females show greater OT FOS colocalization in the PVN following a 10-minute social interaction compared to no social interaction controls. E) Representative SON image, F) SON of a male hamster, G) SON of a female hamster. H) Both males and females show greater OT FOS colocalization in the SON following a 10-minute social interaction compared to no social interaction controls. (* indicates significant difference between groups, $p<0.05$)

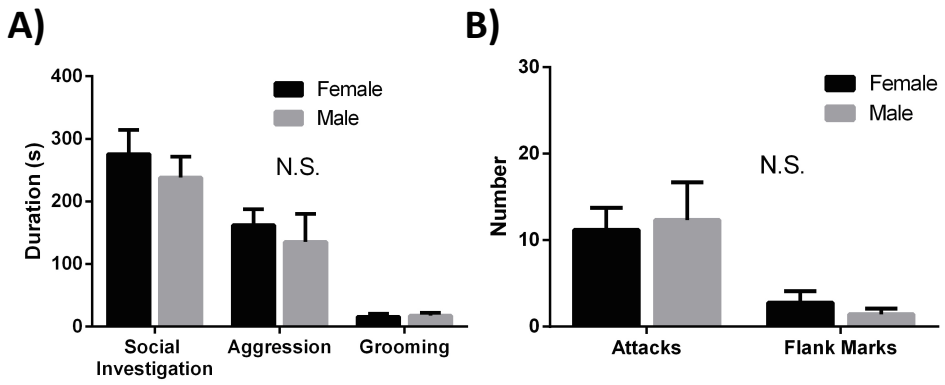


Figure 4.12: Social behavior in males and females during a ten minute social interaction test prior to the determination of the colocalization of oxytocin-and cfos-immunoreactivity.

A) There were no sex differences in the duration of social investigation ($t(16) = 0.735$, $p=0.473$), aggression ($t(16) = 0.517$, $p=0.612$) or grooming ($t(16) = -0.240$, $p=0.813$) during the 10 minute social interaction test. B) There were no sex differences in the number of attacks ($t(16) = -0.221$, $p=0.828$) or flank marks ($t(16) = 0.895$, $p=0.384$) in females and males.

4.5 Discussion

These data provide the first evidence that same-sex social interactions are more rewarding in females than in males in an animal model. This finding is consistent with studies in humans that women find positive social interactions with same-sex partners to be more rewarding than men do (Feng et al., 2015). Further, these studies demonstrate that activation of OTRs in the VTA play a critical role in mediating the rewarding properties of social interactions in females as well as males. Support was also provided for the hypothesis that there is an inverted U-shaped relationship between the duration of social interaction and social reward, mediated by OT within the VTA in both males and females, and that this dose-response relationship is initiated at lower doses in females than males. As predicted, when males and

females experienced the same "dose" of social interaction (i.e., 3 X 10 min sessions), 9μM OT in the VTA *reduced* social reward in females, but *increased* it in males. When the dose of social interaction was reduced (i.e., one 10 min session), however, intra-VTA OT in females *increased* social reward. Despite these sex differences in the response to OT in the VTA, no sex differences in the number of OT-containing neurons activated by same-sex social interactions appeared in either the PVN or SON, two of the most prominent sites of OT projection into the VTA.

We have presented an inverted U-shaped hypothesis as a heuristic tool with which to consider how valence, both positive and negative, is assigned to social stimuli in a sex-dependent manner (Borland et al., 2019a). This hypothesis proposes that as the dose (i.e. duration or intensity) of social interactions increases, their rewarding nature is initially increased, but ultimately reduced. It includes the proposal that this relationship is initiated at lower intensities of social interactions in females compared to males. The utility of this hypothesis for understanding social reward will depend, at least in part, on the ability to define more clearly the critical elements of the duration or intensity of social interactions that are responsible for their rewarding properties. Parametric relationships between the duration of social interactions and its rewarding properties have been identified (Borland et al., 2018) and appear to be similar to at least some of the parametric relationships between drug dose and drug reward (Maldonado et al., 1993; Doherty et al., 2013). In contrast, while the notion of an inverted U relationship between the intensity of social interactions and social reward is intuitively appealing, the concept of social intensity needs to be more fully developed. For example, whether social intensity increases by the numbers of animals engaged in the interactions, the arousal levels of those animals engaged, and/or the degree of emotional involvement in the social interaction, remains unknown.

Importantly, the inverted U hypothesis also proposes that the relationship between the dose of social stimuli and their reward value is mediated by the activation of OTRs in the mesolimbic dopamine system. Data consistent with an inverted U dose-response relationship for the effects of OT administered systemically or directly within the VTA have been reported previously. Administration of OT into the VTA in male rats stimulates penile erections when given at intermediate doses, but not when administered at lower or higher concentrations (Melis et al., 2007). In female mice, OT injected centrally induces a conditioned place preference for female stimulus mice at intermediate doses, but this preference is lost at higher concentrations (Kent et al., 2013). Further support for a sex-dependent inverted U dose-response relationship between OT and social reward comes from studies employing intranasal administration of OT. In female mice, pairing intranasal administration of OT (12 μg) and interactions with another female mouse induces a social place preference for the stimulus mouse, but if the concentration of OT is increased to 36 μg, the initial preference for the female paired with OT is lost; the stimulus mouse becomes aversive (Kosaki and Watanabe, 2016). In contrast, in male mice, pairing of intranasal administration of OT (12 μg) with another male mouse does not induce a social place preference, thus supporting the hypothesis that the dose-response relationship between social reward and OT is initiated at higher doses in males than in females.

Studies in male rodents have established a critical role of OTRs in the VTA in activating dopamine-containing neurons (Xiao et al., 2017) and in mediating social reward (Song et al., 2016; Hung et al., 2017). The mechanisms underlying the sex differences in the response to activation of OTRs are not known but may involve sex differences in the mesolimbic dopamine system (for a review (Gillies et al., 2014)). For example, basal extracellular levels of dopamine in the NAc are higher in female rats compared to males (Virdee et al., 2014), and females display

a faster rate of dopamine uptake and release than males (Walker et al., 2000). In addition, pharmacological or electrical stimulation leads to greater dopamine efflux in the NAc of female rats compared to males (Walker et al., 2000; Walker et al., 2006). Social stimuli can also trigger greater dopamine release in females than males, as shown in rats exposed to same-sex stimulus animals after a period of social isolation (Grotewold et al., 2014). In female rats, the levels of the dopamine metabolite DOPAC in the striatum were higher during same-sex social interactions, whereas in males no changes in DOPAC levels were observed (Weiss et al., 2015). Similar sex differences have also been found in humans (Mozley et al., 2001). For example, women have a higher synaptic concentration of dopamine in the striatum than men (Laakso et al., 2002), as well as a stronger response in the ventral striatum to prosocial decisions (Ross et al., 2017). Therefore, it seems likely that interactions between the activation of OTRs and dopamine play a critical role in mediating the sex differences in social reward. An alternative possibility may be that oxytocin's sex dependent effects on social reward may be due to sex differences in the neuroendocrine profiles associated with aggressive behavior. For example, male and female California mice exhibit different neuroendocrine responses to aggression (Soutschek et al., 2017; Oyegbile and Marler, 2005). Yet previous studies, although not comprehensive, have not revealed sex differences in adrenocorticotropin or cortisol levels related to agonistic encounters (Trainor et al., 2010) or social housing (Huhman et al., 2003) in Syrian hamsters.

A key function of the social reward circuitry is the appropriate assignment of positive and negative valence to social stimuli. In many psychiatric and neurodevelopmental disorders, however, the actual or perceived valence of social stimuli normally perceived as rewarding is attenuated, and, in some cases, even reversed. The inverted U hypothesis provides a simple

proposal for understanding how dose of social interactions may determine reward value that can be used to investigate the mechanisms that mediate it. Further, because the inverted U hypothesis proposes that the dose-response relationship between dose and social reward is initiated at lower doses in females than males, this hypothesis may provide a basis with which to investigate the substantial sex differences in many features of psychiatric and neurodevelopmental disorders. Indeed, the OTR containing circuits in the mesolimbic dopamine system may represent a target of opportunity for treating a diverse group of disorders including substance abuse, autism spectrum disorders and schizophrenia.

5 CONCLUSION/SYNTHESIS

Note: parts of this conclusion/synthesis have been published with contributions from co-authors: James K. Rilling, Kyle J. Frantz, H. Elliott Albers and anonymous reviewers

(Borland et al. 2019a)

5.1 Sex differences in social reward

Many different types of social interactions are rewarding (i.e., defined by their ability to elicit approach responses (White, 1989)) and the neural mechanisms mediating social reward play an essential role in social motivation (Trezza et al., 2011; Panksepp and Yovell, 2014). Indeed, the powerful rewarding properties of social interactions are evident even in species that are not overly gregarious (Gil et al., 2013). Social reward is critical for the formation and maintenance of adaptive social relationships such as pair bonding and dominate/subordinate relationships (Gingrich et al., 2000; Young and Wang, 2004a; Gray et al., 2015; Greenberg et al., 2015). Most of what we know about social reward has come from studies of males. However, even though social interactions have been found to be rewarding in both males and females (Douglas et al., 2004). For example, although there are data indicating sex differences in the rewarding properties of various stimulus modalities (e.g., food and drugs), little is known about whether there are sex differences in the rewarding properties of social interaction. We report here for the first time in a rodent model that females find same-sex social interactions more rewarding compared to males. Using both classical and operant conditioning methods, we demonstrate that same-sex social interactions are more rewarding in female hamsters than in males even though the social behaviors observed during these same-sex social interactions are quite similar in both sexes (Figure 4.3). In a conditioned place preference (CPP) test, females spent about twice as much time in the chamber associated with a same-sex stimulus hamster, as

males spent in the chamber with a same-sex stimulus hamster. Similarly, in a novel Operant Social Preference test, the reinforcing properties of social interactions were significantly greater in females compare to males. Specifically, hamsters were placed in a three-chambered apparatus and allowed access to either a chamber containing an unrestrained same-sex stimulus hamster or an empty chamber, accessed through one-way entry, vertical-swing doors. Females made about twice as many entries into the chamber containing the stimulus hamster as did males. This is also evidence to suggest that women find positive social interactions with same-sex partners to be more rewarding than men do (Feng et al., 2015a).

While much more needs to be learned, these data suggest that more comprehensive studies of sex differences in social reward may be essential for defining the basic mechanisms underlying many different types of social behavior. In addition, this knowledge has significant translational importance, as several lines of evidence point to deficits in social reward as one of the central symptoms and causes of psychiatric disorders, such as autism spectrum disorder, antisocial personality disorder and attention deficit disorder. The prominent sex differences in the incidence of these and other psychiatric disorders (Ramtekkar et al., 2010; Dichter et al., 2012b; Stavropoulos and Carver, 2013; Novacek et al., 2016) may be based in part on sex differences in social reward. Therefore, sex differences in social reward and its underlying neural mechanisms are critical areas of focus for future research.

5.2 Parameters regulating social reward in males and females

To evaluate the parameters regulating social reward in animal models, we explored the relationship between the reward value of social interactions (e.g., duration of interaction) and the frequency of choosing social interaction and found that it mimics the relationship between the reward value of drugs (e.g., dose of drug) and the frequency of drug intake (Figure 3.2). In both

cases, as the reward value increases, the number of rewards obtained in a test session decreases (Maldonado et al., 1993; Doherty et al., 2013). Another well-known relationship drawn from the literature on rewarding properties of drugs is the inverted U-shaped dose-response curve between drug dose and reward value (Uhl et al., 2014). Initially, as dose increases, reward value also rises, but only to a point. Once this peak is reached, increasing drug dose further begins to drive down the reward value. We raise the possibility here that a similar relationship exists between the "dose" of social interaction and the value of the social reward. In this case, the "dose" of social interaction might be defined by duration (e.g. time spent in an environment where social interaction is possible) or intensity (e.g. number of conspecifics available for interactions, or time since last social interaction). The concept of an inverted-U function between the duration or intensity of social interaction and its rewarding properties may provide a framework in which to describe how social stimuli can transition from positive to negative valence, as in a variety of psychiatric disorders, such as social anxiety, agoraphobia, enochlophobia, and autism spectrum disorder. If so, as duration and/or intensity of social interactions increase, the rewarding properties of those interactions would be initially increased, then ultimately reduced. Consistent with this possibility are the findings in rats that brief social interactions are more rewarding than longer interactions (Bardo et al., 2013; Zernig and Pinheiro, 2015), and that rodents housed in social isolation find brief social interactions more rewarding than do rodents housed in groups, where social interactions occur continuously (Douglas et al., 2004; Matthews et al., 2005). Because females appear to be more sensitive to the rewarding properties of social interaction than males, the inverted U function would be displaced such that less social interaction would be required in females to produce the same levels of social reward, compared to males. The differences in the inverted U functions in males and females may be useful in predicting sex

differences in the responses to social interactions, and as a guide to understanding how the neural mechanisms that mediate social reward differ in males and females. Indeed, one of the major challenges in the development of new treatments for psychiatric disorders is understanding the transition from assigning positive value to social stimuli to assigning negative value to social stimuli, or at least omitting positive value, as in psychiatric disorders that include social impairments. Treatments intended to improve or restore positive social attributions may need to account for these "dose-effect" relationships, and potential sex differences therein.

5.3 Sex differences in OTR regulation of social reward

To investigate if OTRs in the VTA mediate this sex difference in social reward, we treated male and female Syrian hamsters with OT, an OTR agonist, antagonist, or saline in the VTA prior to social interaction training sessions in the CPP paradigm. Injection of a selective OTR antagonist into the caudal VTA significantly reduced the rewarding properties of same-sex social interactions by more than 50% in both males and females, thus supporting the hypothesis that OTRs mediate social reward through their actions in the VTA in both sexes (Figure 4.5). Surprisingly, however, injection of OT itself or a highly selective OTR agonist into the VTA has opposite effects on social reward in males and females (Figure 4.7); whereas the OTR agonist (or OT) in the VTA significantly increases social reward in males, it significantly *reduces* social reward in females. One interpretation of these results is that females are closer to the peak of an inverted-U dose-effect relationship between social interaction and reward value at baseline, and that further elevation of reward value by activation of OTRs with an exogenous agonist pushes reward value past peak levels, thereby causing a decline in reward value. In contrast, because males experience lower levels of social reward at baseline, a further elevation of reward value by activation of OTRs in the VTA simply increases the rewarding properties of the interaction. If

this interpretation is correct, OT would be predicted to enhance the rewarding properties of social interactions in females in situations where the social reward of those interactions had not peaked (i.e. a low "dose" of social interaction). To test this hypothesis, the "dose" of social reward was lowered in females by reducing the number of social interaction trials during CPP from the three used in the prior experiments to a single trial. When social reward was reduced in females using this approach, OT injected into the VTA significantly increased the rewarding properties of that single social interaction trial (Figure 4.7).

To investigate if differences in OT recruitment for same-sex social interactions may contribute to sex differences in social reward, we investigated the effect of a 10-min social interaction on OT neuronal activity in males and females. Same-sex social interactions increased the percent of OT-ir neurons in the PVN and SON (Figure 4.11) co-localized with c-Fos in males and females. Interestingly, no sex differences in the level of OT recruitment for same-sex social interactions were detected in the PVN and SON.

Taken together, the data summarized above suggest that OT in the VTA is a primary neural signal through which social stimuli trigger the mesolimbic DA pathway to assign salience to social interactions, thereby making them rewarding. The data further support the contention that females exhibit higher sensitivity than males to this process, perhaps through more transient levels of DA release in the NAc triggered by social interactions and/or a heightened postsynaptic impact of DA projections to forebrain nuclei such as the NAc.

5.4 Synthesis

As is the case in most areas of social neuroscience, the mechanisms underlying social reward have been investigated more extensively in males than in females. The existing data in rodents and humans, however, suggests that females find same-sex interactions to be more

rewarding than males. Given that activation of OTRs in the VTA and other areas of the mesolimbic circuitry is necessary for social reward processing in both males and females, the sex differences in social reward could result from sex differences in the OT system. As outlined above, sex differences consistent with the behavioral data and effects of OT could be related to endogenous levels of OT, neuronal responses to OT, and/or downstream signaling in the DA reward circuitry. Because OT /AVP can be released locally from pre-synaptic neuronal terminals, as well as more globally from non-synaptic regions of neurons, it is difficult to know if there are sex differences in the amount of AVP/OT reaching OTRs in the mesolimbic system. It is noteworthy, however, that women have higher baseline CSF OT levels (Altemus et al., 1999). Although OTRs have been identified throughout the neural circuitry controlling social behavior, there is no compelling evidence for the presence of higher concentrations of OTRs in females than in males. In fact, the opposite may be true in some regions, as a greater number of OTRs has been reported in subregions of the striatum for males than females in rodents. Nevertheless, possible sex differences in the signaling triggered by OTR binding could exist, such as in sex differences in the effectiveness of coupling to G-proteins or the specific array of G-proteins available for coupling. Another possibility is that OT activation within the mesolimbic system is not sexually differentiated, but the higher basal or transient DA activity in females compared with males is the explanatory mechanism.

Furthermore, differences in social reward might not be due to differences in responsiveness or effects of the OT system, but sex differences in the responsiveness and effects of the DA system. For example, there may be differences in the concentration and region specific release of DA between males and females. DA release in the prefrontal cortex (PFC) may signal aversion (Lammel et al. 2011). Males may initially have greater DA release in the

PFC for social interactions compared to females, resulting in a blunted reward signal. Another possibility is that females may find social interactions more rewarding than males not because of differences in DA recruitment or release, but due to increased sensitivity to DA release. Previous studies indicate membrane bound estrogen receptors can sensitize both mGluR and DA receptors in the NAc (Micevych and Mermelstein, 2008, Song et al., 2019). Thus, estrogen receptors in the NAc may play a role in the increased sensitivity of females to drugs of abuse.

Based on data from rodents and humans we propose that there is an inverted U relationship between the duration and/or intensity of social stimuli and social reward value, perhaps modulated by OT in the VTA, and possibly other regions of the mesolimbic DA system. Further, we propose OTR activation in the mesolimbic DA system is necessary for the rewarding properties of social interactions in both males and females. However, the inverted U relationship between OT dose, social reward and neural activity is initiated at lower doses in females than males. As a result, depending on the dose of OT administered, OT could enhance social reward in males while reducing it in females. Although the hypothesis of an inverted U dose-response relationship for the effects of mesolimbic OT is speculative, precedence exists for an inverted U dose-response function for the effects of OT. Previously, an inverted U dose-response for OT was identified following systemic administration or injection of OT directly into the VTA (Popik et al., 1992; Boccia et al., 1998; Melis et al., 2007). For example, injections of OT into the caudal VTA of male rats induce penile erections when given at intermediate concentrations (i.e., 40 or 80 ng) but not lower (i.e., 20 ng) or higher concentrations (i.e., 100 ng). The potential cellular and molecular mechanisms underlying an inverted U dose-response are not known. One possibility is that as OT concentrations increase they activate AVP receptors in addition to OTRs and the activation of AVP receptors reduces the effects of OTR activation. This possibility may

be unlikely, however, because administration of concentrations of OT that activate V1a AVP receptors (90 μM OT) do not reduce responses mediated by OT activation of OTRs (Song et al., 2014; Song et al., 2016b). Another interesting possibility, however, relates to the finding that the coupling of OTRs to different G proteins can result in different, or even opposing effects within the cell (Gravati et al., 2010). Because the concentration of OT determines the coupling of OTRs to different G protein subtypes (Busnelli et al., 2012) perhaps the inverted U shaped dose-response might be the result of concentration-dependent effects on the coupling of OTRs to different G protein subtypes.

Although the rewarding properties of many types of social interaction are self-evident, the importance of social reward in the expression of adaptive and maladaptive behavior, and in the establishment and maintenance of social relationships remains to be fully appreciated. There is increasing evidence that dysfunctions in the mechanisms mediating reward can play a substantial role in the expression of a large number of psychiatric disorders, including substance-abuse, affective disorders and obsessive-compulsive disorders as well as in a range of neurodevelopmental disorders including autism spectrum disorder, schizophrenia and attention-deficit/hyperactivity disorders. Indeed, it has been proposed that dysfunctions in the circuits mediating reward (e.g., mesolimbic DA system) may be present in many different psychiatric and neurodevelopmental disorders and represent a common target for their treatment (Dichter et al., 2012a). Recently, the National Institute of Mental Health has developed the Research Domain Criteria (RDoC) as a "research framework for new approaches to investigating mental disorders" and has defined two of these domains as "Negative Valence Systems" that are primarily responsible for responses to aversive situations and "Positive Valence Systems" that are primarily responsible for responses to positive motivational situations. Not infrequently,

however, a characteristic of mental disorders is that social stimuli that normally have a positive valence can become less rewarding or even aversive. It is interesting to consider whether an inverted U function of the rewarding properties of the social duration and/or intensity of social interactions could contribute to changing the valence of social stimuli from positive to negative, and thereby contribute to the symptomology of mental disorders. One possibility is that the perceived value of social interactions could be abnormally increased or decreased in specific disorders. If so, the direction and magnitude of the change in perceived value of social interaction would determine the sex-dependent change in the valence of social stimuli.

While there are substantial sex differences in the incidence of many psychiatric disorders relatively little is known about the underlying causes of these differences (Cover et al., 2014; Gobinath et al., 2017), although it seems likely that sex differences in the rewarding properties of social interactions is a likely contributor. For example, the possibility that social interactions are less rewarding in men than women at baseline could pre-dispose men to be more susceptible to disorders such as autism which is characterized by diminished social motivation and reward, and which does indeed occur four times more frequently in men than women (Ferri et al., 2018). It also seems likely that as more research is conducted in females, more sex differences will be found in the factors that contribute mental disorders. For example, social stress is a significant factor in many types of mental disorders, and it is now clear that females are more susceptible to social stress than males (Bangasser and Valentino, 2014). Perhaps an oversensitive social reward system pre-disposes women to be more susceptible to social stress disorders. Taken together, these data suggest that the causes of mental disorders such as stress and a diminished capacity for social reward may interact in complex ways that differ in men and women and indicate the importance of the development gender-based treatments.

OT has been proposed to be a promising treatment for a wide range of psychiatric disorders including substance abuse, autism spectrum disorders, anxiety, stress-related disorders and schizophrenia (McGregor and Bowen, 2012; Rich and Caldwell, 2015; Gottschalk and Domschke, 2017; Sippel et al., 2017; Benner and Yamasue, 2018). If the inverted U hypothesis of a sex-dependent relationship between OT dose and its effects on DA signaling and social reward (and perhaps other factors important in the etiology of mental disorders) is correct, then consideration of gender differences in OT administration will be particularly important. Indeed, giving the same dose of OT in men and women could have the opposite effect producing a positive clinical outcome in one sex while producing a negative clinical outcome in the other. While considerable effort is underway to develop drugs that can act selectively on OTRs in the brain, at present the most common route of administration of OT in humans is intranasal. Intranasal OT administration is thought to produce supraphysiological levels (Leng and Ludwig, 2016), making it particularly important to examine the dose-dependent effects of OT in both men and women.

5.5 Future area of research

Because social reward plays such a key role in the expression of social behavior, more comprehensive studies of the rewarding nature of social interactions and their neural mechanisms in males and females are needed. In particular, it will be important to define what specific characteristics of social interactions alter the rewarding properties of those interactions. For example, while the *duration* of social interactions have been shown to alter reward value, the conditions under which the *intensity* of social interactions might alter their reward value are less clear. It will also be important to determine how the rewarding properties of different types of social interactions may differ in males and females. For example, is aggression more rewarding

in males than in females in some species? In addition, some types of social interactions may elicit different states of arousal in males and females resulting in social experiences that are qualitatively different. Particularly in humans same-sex interactions may be more complex and represent more than just sex differences in the rewarding properties of those interactions. For example, while same-sex social interactions could also be influenced by motivation to avoid agonistic social interactions, which may have more severe consequences for one sex than the other (e.g., the more aggressive sex). If so, OT might influence social behavior by modulating the impact of negative social interactions. For example, OT has been shown to increase trust among men in an economic game, presumably by decreasing the threat of betrayal, as inferred from attenuated amygdala activation (Baumgartner et al., 2008).

Investigation of the role of the AVP/OT family of peptides in social neuroscience will likely continue at a rapid pace because of its importance for understanding the basic neural mechanisms of social behavior and their translational significance. Understanding the action of these peptides will require studies of the functional significance of the cross-talk between OT and AVP and their receptors in both males and females. It will also be important to define the critical sex differences in AVP/OT signaling. What are the roles of sex differences in the amount and distribution of peptide release (e.g., synaptic and non-synaptic release), the number and distribution of their receptors and/or in the cellular and/or network events precipitated following receptor activation? It will also be important to determine the role of gonadal hormones in mediating sex differences in social reward, particularly because they play an important role in sex differences in drug reward.

Another key area of future research will be to define the dynamic interactions between OT and the many other neurochemical signals found within the mesolimbic DA system that

contribute to reward, and how these interactions differ in males and females. Of course, interactions with DA will be of central importance but in addition it will be critical to determine the roles of the many other neurochemical signals likely play an important role such a serotonin, GABA and glutamate. It will be interesting to fully test the inverted U hypothesis of the rewarding properties of the duration/intensity of social interactions and determine the extent to which they can account for changing the valence of social stimuli from positive to negative (or vice versa) thereby contributing to the symptomology (and potentially treatments) of mental disorders in men and women. If the inverted U hypothesis of a sex-dependent relationship between OT dose and its effects on social reward and perhaps other factors important in the etiology of mental disorders is correct then consideration of gender differences in OT administration will be particularly significant. It will be particularly important to examine the effects of OT dose on social reward as well as its utility in developing gender-specific OT treatments for a range of psychiatric and neurodevelopmental disorders.

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